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## Interactions between potato plants, arbuscular mycorrhizal fungi, potato cyst nematodes and a nematicide

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Interactions between potato plants, arbuscular mycorrhizal  
fungi, potato cyst nematodes and a nematicide

By

Thomas Deliopoulos

(B.Sc., M.Sc.)

Thesis submitted in partial fulfilment of the requirements for the degree of  
Doctor of Philosophy of the UK Open University

Crop and Environment Research Centre, Harper Adams  
University College, Newport, Shropshire, UK



&

Department of Zoology, Ecology and Plant Science,  
National University of Ireland, Cork, Ireland



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*To my Parents*



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## Abstract

Inoculation of potato plants with Vaminoc, a commercial mixture of three selected isolates of arbuscular mycorrhizal fungi (AMF), and with two of these isolates, *Glomus intraradices* (BioRize BB-E) and *Glomus mosseae* (isolate BEG 12) increased the early *in vitro* (in potato root leachate, PRL) and in soil hatch of the potato cyst nematode (PCN) *Globodera pallida* (0-4 weeks after shoot emergence), but had no effect on the hatch of the other PCN species *Globodera rostochiensis*. In the absence of AMF, *G. pallida* exhibited delayed *in vitro* and in soil (microplants or plants grown from tubers) hatch, compared to *G. rostochiensis*; inoculation of potato roots with AMF eliminated this delay in *G. pallida* hatch. While the effects of these AMF isolates on PCN hatch were consistent across a range of potato cultivars, the effect on plant growth was more variable, with certain AMF-cultivar associations being more effective than others on plant growth enhancement. The growth parameter mostly affected (increased) by mycorrhizal inoculation was root growth. When the PRL from AMF-untreated and AMF-treated plants were fractionated and corrected for carbon content, it was revealed that mycorrhizal inoculation had multiple effects on hatching factor (HF) production: generation of novel HFs (more evident with Vaminoc than with the single AMF isolates), up or down regulation of existing HFs. The result of the AMF-PCN interaction depended on the time that mycorrhizal inoculation took place: pre-inoculation with AMF 2 weeks before planting followed by one more AMF application at planting significantly lessened the multiplication of *G. pallida* on the roots, relative to AMF-untreated plants; in contrast, one application of AMF (at planting) increased the final *G. rostochiensis* population size (but not *G. pallida*), compared to AMF-untreated plants. In the presence of the nematicide aldicarb, the multiplication rate of *G. pallida* was significantly lower on roots inoculated with AMF than on roots that did not receive mycorrhizal treatment at planting.

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**Chapter A**  
**Literature Review**

## A.1 Potato cyst nematodes

### A.1.1 Introduction

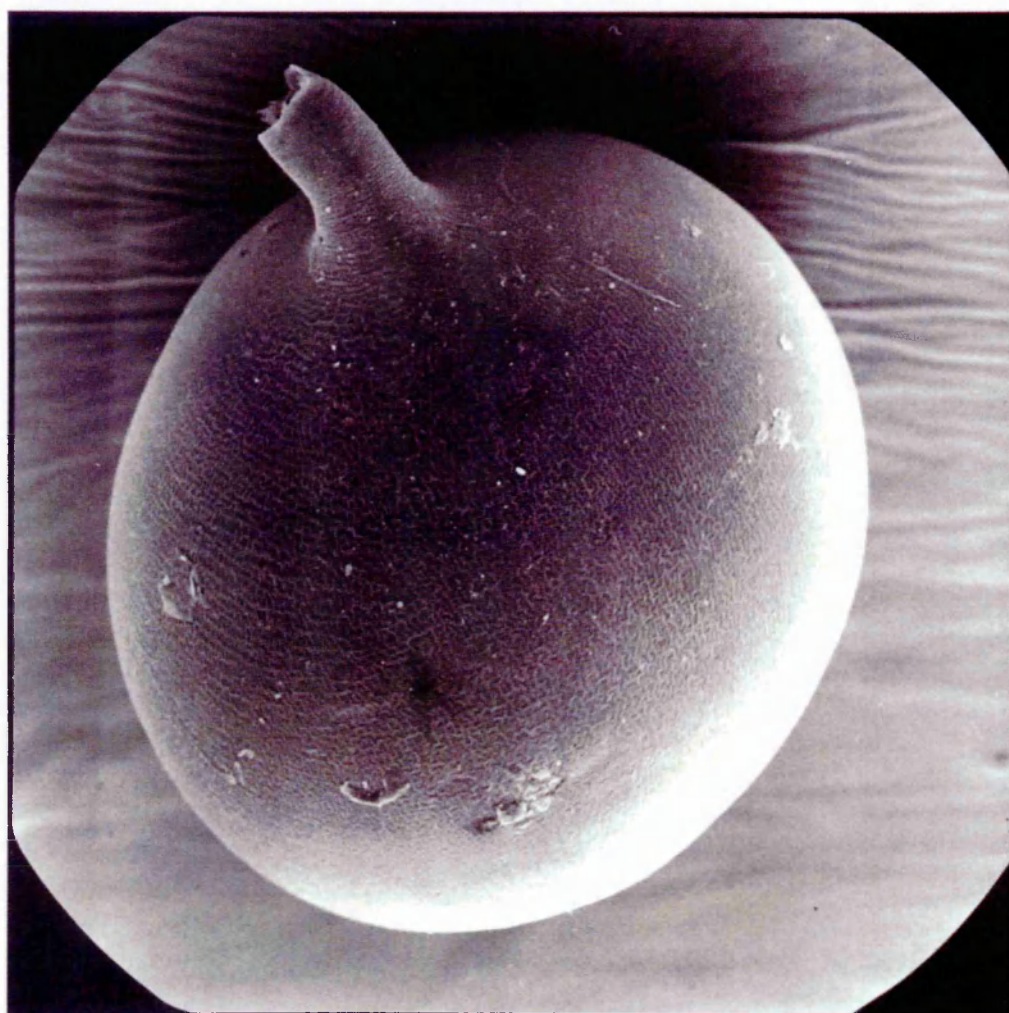
Nematodes (GK, nema, nematos = thread; eidos = form) are one of the most abundant groups of invertebrates, occurring in almost all environments. They are also referred to as eelworms, threadworms or roundworms. Some nematodes are free-living organisms, feeding on microorganisms such as fungi and bacteria, while others parasitise animals (including humans) and plants. Approximately 2000 species of nematodes occur in soil. The majority of soil-borne nematodes live saprophytically on decaying or dead organic matter, while approximately 500 species are plant parasites causing a variety of diseases worldwide (Hooper, 1973). The majority of plant parasitic nematodes feed on roots, but some feed on stems, buds or leaves. The annual yield losses caused by nematodes worldwide on economically important crops (including vegetables, fruits and nonedible field crops) are estimated to be approximately 14%, corresponding to losses of over US \$80 billion in annual crop value (Agrios, 1997).

Cyst nematodes (genera *Globodera*, *Heterodera* and *Punctodera*) are the most highly adapted plant parasitic nematodes in temperate regions of the world (Whitehead, 1998). They derive their name from the female nematode which dies after fertilisation is complete, forming a hard round covering surrounding the eggs, known as a 'cyst' (Fig. A.1). Some species of cyst nematodes attack only one or a few plant species and are limited to certain geographical areas, whereas others have a very wide host range and distribution. The most important cyst nematode species and their hosts are listed in Table A.1.

The two species of potato cyst nematodes (PCN) *Globodera pallida* (Stone, 1973b) and *G. rostochiensis* (Wollenweber, 1923), are distinct but closely related,



and are highly destructive pests of the potato crop in most potato-growing areas throughout the world, except from countries where stringent quarantine regulations are enforced, as for example in the USA and Canada where PCN has been confined to New York (*G. rostochiensis* only present; Brodie & Mai, 1989; United States Department of Agriculture) and a relatively small geographical area of Newfoundland only (both species present; Department of Agriculture, Fisheries and Aquaculture, Canada), respectively.



**Fig. A.1.** Mature cyst of potato cyst nematode (x100 magnification)

**Table A.1. List of important cyst nematode species and their hosts  
(adapted from Stone, 1986)**

<u>Species</u>	<u>Common name</u>	<u>Hosts</u>
<b><i>Globodera</i></b>		
<i>G. pallida</i>	Potato cyst nematode (white)	Potato, tomato, aubergine
<i>G. rostochiensis</i>	Potato cyst nematode (golden)	Potato, tomato, aubergine
<i>G. tabacum tabacum</i>	Tobacco cyst nematode	Tobacco
<b><i>Heterodera</i></b>		
<i>H. schachtii</i>	Sugar beet nematode	Sugar beet, spinach
<i>H. avenae</i>	Cereal cyst nematode	Wheat, barley
<i>H. trifolii</i>	Clover cyst nematode	Clover
<i>H. carotae</i>	Carrot cyst nematode	Carrots
<i>H. cruciferae</i>	Cabbage cyst nematode	Brassicas
<i>H. glycines</i>	Soybean cyst nematode	Soybean
<i>H. oryzae</i>	Rice cyst nematode	Rice
<b><i>Punctodera</i></b>		
<i>P. punctata</i>	Grass cyst nematode	Grass

### **A.1.2 Origin and distribution**

Potato cyst nematodes, together with their primary host, the potato, are thought to have originated in the Andean regions of South America (Greco, 1988). The potato, which, after maize is the most widely distributed crop in the world, was first introduced in Europe as a curiosity in the 16th century (Beukema & Van Der Zaag, 1990). The equatorial South American days are short and consequently, the first cultivated potatoes that were transplanted to Europe did not start to produce tubers until autumn and therefore, tubers did not achieve a significant size. There was therefore a need to discover cultivars which would flower under long day conditions so that tuber production would start in the summer and higher yields would be achieved (Beukema & Van Der Zaag, 1990). As a result, breeding programmes for

adaptation started; genes that conferred selective advantages to the local conditions predominated and were used for the development of new cultivars (Rolfe, 2003). By the 18th century, as potato plants became eventually adapted to the prevailing long days during the growing season, potato was already an important food crop in Europe, especially for the poorer regions of the continent. It is speculated that PCN reached Europe in the 1840s when the potato famines, caused by late blight, led to the importation of large quantities of tubers from South America for breeding purposes in order to develop blight-resistant varieties (Jones, 1970). Most commercial potato cultivars are *Solanum tuberosum* var. *tuberosum* and are derived from the original *andigena* group *S. tuberosum* var. *andigenum* (*S. stenotomum* x *S. sparsipilum* hybrid; Hawkes, 1990).

In Europe, the first recording of field damage by PCN was in Germany in 1913 and the most recent new record was in Yugoslavia in 2002 (Hockland, 2002). The two PCN species have been recorded in nearly all countries in Europe. In contrast with western Europe, in many central and, in particular, eastern European countries, despite the fact that potato is also a crop of major significance, less official data are available on the distribution of the two PCN species and the rate of PCN spread and according to Hockland (2002), these are only indicative of when surveys were conducted or when damage was first recorded. Furthermore, up to 1973 all PCN were thought to be *Heterodera* (*Globodera*) *rostochiensis* (Section A.1.4). These are probably the reasons why *G. pallida* is not displayed in PCN distribution lists (EPPO/CABI, 1997; CABI, 2000; EPPO, 2000) in the following countries in Europe: Albania, Belarus, Bulgaria, Croatia, Czech Republic, Estonia, Finland, Latvia, Liechtenstein, Lithuania, Russia, Slovakia and the Ukraine. Formal surveys will need to be conducted with sampling of large number of PCN-infested fields and

identification of PCN to species level to investigate whether *G. pallida* is absent from these countries. Such a strategy has been adopted by the European Commission in the new Plant Health Directive (2000/29/EC; Anon., 2000). The Directive requires that EU Member States shall take measures against *G. pallida* and *G. rostochiensis*, which, amongst other things, shall include official surveys in order to investigate and confirm the presence of the two PCN species and determine their distribution. Hungary for example, was also included up to 2000 in the same PCN distribution lists with no *G. pallida* occurrence but a recent country-wide *Globodera* monitoring programme revealed the presence of *G. pallida* in some PCN-infested fields (Palkovics, 2003). The only European country that is certainly free from *G. pallida* is Finland since it has Protected Zone status for the species *G. pallida*, the reason being the effective implementation of quarantine regulations described in the previous Plant Health Directive 77/93/EEC (Whitehead & Turner, 1998), which prevented the entry of this pest in the country (Anon., 2000). In Greece and Denmark the occurrence of *G. pallida* is localised and there are only reported interceptions, not field infestations with *G. pallida* (Hockland, 2002); according to Jones & Jones (1984) in countries where *G. pallida* (or *G. rostochiensis*) is confined to small areas, strict quarantine programmes within the country can help prevent further spread.

PCN-resistant cultivars in Europe usually contain the H<sub>1</sub> (ex *S. andigena*) gene. Such cultivars are effective against *G. rostochiensis* pathotype Ro1 which lacks the gene for virulence against the H<sub>1</sub> gene, but they are ineffective against *G. pallida* pathotypes Pa2 and Pa3. The continuous cultivation of these *G. rostochiensis*-resistant cultivars has resulted in *G. pallida* being now the predominant species in many potato-growing regions in Europe. In England and Wales, in particular, about 64% of the land cropped with potatoes is infested with PCN, with *G. pallida* being

the predominant species (of the 311 PCN-infested sites, 67% were pure *G. pallida*, 8% were pure *G. rostochiensis*, and 25% contained a mixture of the two PCN species) (Minnis *et al.*, 2002). In contrast, a similar survey conducted in Northern Ireland in soil samples from 80 PCN-infested sites revealed that *G. rostochiensis* was the predominant species (pure *G. rostochiensis*: 81.25%, pure *G. pallida*: 4%, mixed *G. pallida* and *G. rostochiensis*: 13.75%) (B. Moreland, personal communication, cited in Devine, 2000). In the Republic of Ireland, *G. rostochiensis* was thought by Jones & Jones (1984) to be the dominant species, but no formal survey has been published to date to confirm that. In the Andean regions of South America, Bolivia, Venezuela and south Peru have mixed populations of *G. pallida* and *G. rostochiensis*. Potato cyst nematode-infested land in Colombia, Ecuador and most of Peru contains pure *G. pallida*, whereas in Chile the only PCN species present is *G. rostochiensis* (Whitehead, 1998).

### A.1.3 Host range and dispersal

Potato cyst nematodes have a very narrow host range feeding solely on plants of one family, the Solanaceae (Williams, 1978). Their preferred host is potato (*Solanum tuberosum*), but they can also attack tomato (*Lycopersicon esculentum*) and aubergine (*Solanum melongena*) (Jones *et al.*, 1998). In addition, PCN have numerous wild solanaceous hosts, such as bitter nightshade (*S. dulcamara*), black nightshade (*S. nigrum*), hairy nightshade (*S. sarrachoides*), jimsonweed (*Datura stramonium*), and silverleaf nightshade (*S. elaeagnifolium*) (Goodey *et al.*, 1965). According to Doncaster (1953), *S. nigrum* may produce leachate capable of stimulating PCN hatch but is a poor host for the nematode.

There are many different ways by which PCN can be introduced and spread within clean fields. Cysts adhered to tubers or present in soil around the tuber can be one mean of PCN spread. Soil residues from PCN-infested fields adhering to farm machinery, implements or boots can also carry cysts. Other routes of PCN spread include wind-blown infested soil, birds, and transplanting of plants from contaminated soils.

#### A.1.4 Classification

All plant parasitic nematodes belong to the kingdom Animalia (Agrios, 1997), phylum Nematoda (Coomans, 1975) and class Secernentea (Hooper, 1978). The majority of these plant parasites, including PCN, belong to the order Tylenchida, suborder Tylenchina (Chitwood & Chitwood, 1950), but a few belong to the order Dorylaimida (families Longidoridae and Trichodoridae; Agrios, 1997). Golden (1971) proposed a super-family Heteroderoidea and Hooper (1978) the family Heteroderidae to which PCN were assigned. All cyst nematodes, including PCN, were initially classified in the genus *Heterodera* (Hesling, 1978). In the early 1970s, nematologists noticed heterogeneity in the morphology between juveniles of *H. rostochiensis* (Stone, 1972) and in the colour between mature females of this species (Guile, 1970) and subsequently Stone (1973b) identified a second PCN species which he named *Heterodera pallida*. Up to this time, all PCN were thought to be of one species, *Heterodera rostochiensis*. Mulvey & Stone (1976) reorganised cyst nematodes into three different genera, *Heterodera*, *Globodera* and *Punctodera* and the two PCN species were re-classified in the genus *Globodera* (Behrens, 1975; Mulvey & Stone, 1976).

### A.1.5 Pathotypes and diagnosis

Pathotype classification in PCN is based on the ability of the nematodes to reproduce on resistant cultivars. The two PCN species have been subdivided into pathotypes that differ in their host range. Kort *et al.* (1977) identified five pathotypes in *G. rostochiensis* (Ro1, Ro2, Ro3, Ro4 and Ro5) and three in *G. pallida* (Pa1, Pa2 and Pa3). According to Whitehead (1998), this classification scheme, although satisfactory for distinguishing pure populations of each pathotype, is impractical when unknown mixed populations are present, as is often the case with field populations. Trudgill (1985) suggested that the variation, observed by Kort *et al.* (1977), in virulence of *G. pallida* on resistant potato cultivars was an artefact, resulting from the introduction of different populations and gene-pools from South American pathotypes. Furthermore, Stone *et al.* (1986) was unable to distinguish between Pa2 and Pa3 in field populations. Nijboer & Parlevliet (1990) concluded that only three *G. rostochiensis* pathotypes existed, Ro1 (old Ro1 and Ro4), Ro2 (old Ro2 and Ro3) and Ro5, while *G. pallida* consisted of just one pathotype (Pa2/3 or Pa) with different levels of virulence. More recent research has shown that UK and European populations of *G. pallida* were not distinct and contained three types of ribosomal DNA that may have been the result of population hybridisation (Trudgill, 2000). The same research showed that the mitochondrial DNA (mtDNA) from South American populations of *G. pallida*, such as the P5A population, was so different from that of UK and European populations that the P5A population may be a new species.

Considering the differences that exist in the biology and, consequently, the responses to crop rotation and chemical treatment between the two PCN species, accurate diagnosis of the species present is essential in order to avoid unnecessary

nematicide applications and scheduling of land (Hockland, 2002). The first step in the diagnosis of PCN must be to distinguish *Globodera* from other cyst nematodes that might also be present in the soil. Morphological differences particularly in the cysts, but also in juveniles, may be used to diagnose *Globodera* from cyst nematodes in the genera *Heterodera* and *Punctodera*. For example, *Globodera* species have round cysts, while the cysts of *Heterodera* and *Punctodera* spp. are lemon shaped and pear shaped, respectively (Luc *et al.*, 1988). Examination of the terminal region of the cysts is a useful key in the identification of cyst nematodes (Golden, 1986). *Globodera* nematodes have no vulval cone or egg sac, whereas, in members of *Heterodera*, the vulval cone is present as a terminal protruberance, and an egg sac is present (Golden, 1986).

Although most morphological characters of *G. rostochiensis* do not differ significantly from those of *G. pallida*, the two species may be distinguished from each other by examining the colour of their females. In *G. pallida*, adult females remain creamy white until dying and becoming a brown cyst, in contrast with those of *G. rostochiensis* which pass through a yellow stage. Due to this difference, *G. rostochiensis* is often referred to as the 'golden potato cyst nematode' (Greco, 1988) and *G. pallida* as the 'white potato cyst nematode' (Williams, 1978). However, distinction based on this characteristic is only possible for a very narrow time period, when females pass through the immature and adult stages before becoming cysts. In addition, this method of distinguishing the two PCN species is impractical because it requires lifting of large numbers of plants and examination of the root systems, something that can be extremely time consuming.

In addition, the tail of the *G. pallida* second-stage juvenile (J<sub>2</sub>) is longer than of *G. rostochiensis* (52 compared to 44 µm; Hesling, 1978). Stylet length is 23-24 µm



long in *G. pallida* J<sub>2</sub>s with knobs pointing forward, whereas in *G. rostochiensis* J<sub>2</sub>s it is 19-21 µm long with knobs pointing backward (Franco, 1986). The anal-vulval distance in the adult females of *G. pallida* is shorter than in those of *G. rostochiensis* (Stone, 1973a). Although adult males of *G. rostochiensis* are slightly shorter than those of *G. pallida* (1.08 mm compared to 1.2 mm), they have a greater mean maximum width compared to *G. pallida* (39 µm compared to 28.4 µm; Hesling, 1978). However, discrimination of the two PCN species based on morphological/morphometrical characters of the adult males and juveniles can be difficult (Baldwin & Mundo-Ocampo, 1991) and time consuming. Identification of PCN species still relies on the expertise of nematologists using traditional microscopy techniques.

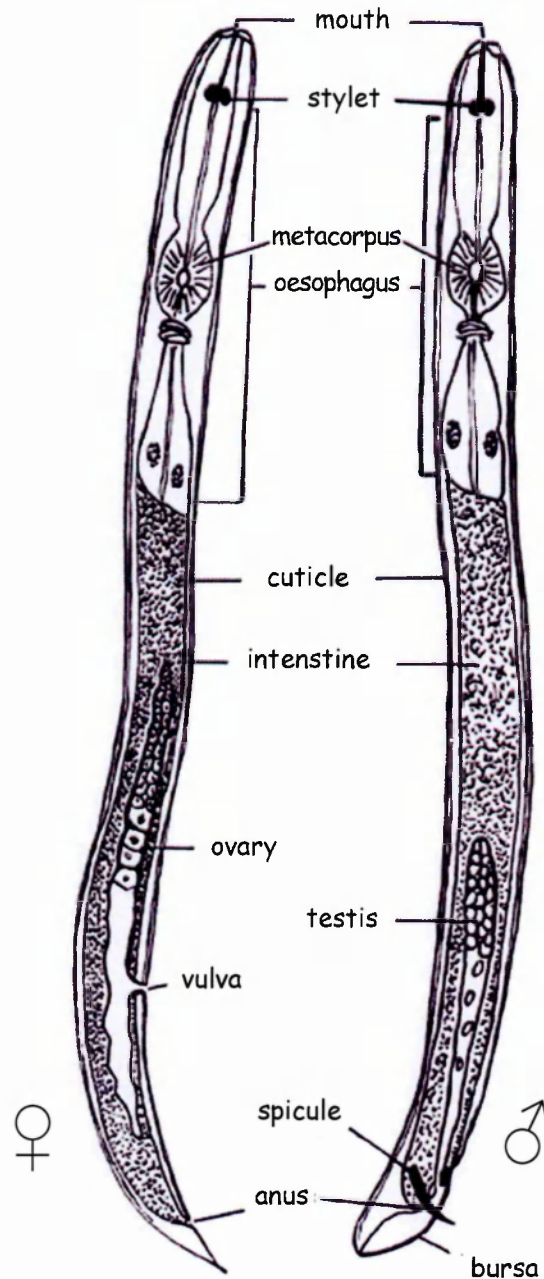
Given this problem, a range of molecular diagnostic techniques have been developed over the last 20 years to distinguish the two PCN species. Some of these methods provide an accurate estimation of the proportion of each PCN species in mixed populations, which is very useful when developing PCN management protocols (Fleming & Marks, 1983). The first report of successful PCN species identification using molecular techniques was that of Trudgill & Carpenter (1971) who separated the two species by polyacrylamide gel electrophoresis (PAGE) on the basis of their protein patterns. Isoelectric focusing technique (IEF) is another method that has been used successfully for discriminating PCN at the species level by locating species-specific proteins (Fleming & Marks, 1982). A powerful DNA-based technique to separate the two PCN species involves the polymerase chain reaction (PCR; Mulholland *et al.*, 1996; Shields *et al.*, 1996). Bates *et al.* (2002) measured the relative proportions of *G. pallida* and *G. rostochiensis* in a mixed PCN sample using a novel approach based on melting peak analysis of PCR products. The sensitivity of

their test was such that they managed to detect a 2% proportion of *G. pallida* cysts in the mixture. Other useful DNA-based methods to distinguish *G. pallida* from *G. rostochiensis* include restriction fragment length polymorphism of ribosomal DNA (rDNA-RFLP; Burrows & Boffey, 1986), southern blotting with diagnostic probes or dot-blotting with specific probes (Marshall, 1993) and random amplified polymorphic DNA (RAPD) techniques (Folkertsma *et al.*, 1994).

At present, none of these techniques is able to distinguish between all native *Globodera* species, which include *G. achilleae* and *G. artemisiae* in northern Europe and *G. tabacum* in southern Europe or between European and South American populations of *G. pallida* which can be very heterogenous (Trudgill, 1985; Blok *et al.*, 1998; Hockland *et al.*, 2000; Greiner *et al.*, 2001).

#### **A.1.6 Morphology and anatomy**

The body structure of a typical female and male plant parasitic nematode can be seen in Fig. A.2. Second-stage juveniles of PCN are circular in cross-section and their body is elongate. The body of the juvenile consists of a body wall, which is divided into cuticle, hypodermis and somatic muscles, surrounding a tube-like gut (oesophagus and the intestine) (Hooper, 1973). The mouth is surrounded by six lips (Bird & Bird, 1991) and behind the mouth is the stylet which is used, with the aid of muscles attached to the posterior knobs, to puncture holes in the egg membrane during hatching and to perforate root cells (Franco, 1986). Nematodes, including PCN, do not have respiratory and circulatory systems (Hooper, 1973). They have however, an alimentary canal extending from the mouth through the oesophagus, intestine, rectum and anus. The oesophageal glands fill the body cavity and contain secretory granules and it is thought that they are involved in the development of the



**Fig. A.2.** Body structure of a typical female and male plant parasitic nematode  
(Credits: C. Jaslavich, the American Phytopathological Society)

grant feeding cell or syncytium (Perry *et al.*, 1989). The reproductive system in the female PCN consists of two ovaries, which are followed by an oviduct and uterus terminating in a vulva (Bert *et al.*, 2002). In the males the reproductive system is monorchic and is composed of a testis, a seminal vesicle, a terminus in a common

opening with the intestine, and a pair of copulatory curve shaped spicules (Stone, 1973a). The males remain elongate until maturity when they twist their body into a C or S shape (Hooper, 1973). Mature males are about 1 mm long (Franco, 1986). Unlike males, upon maturity the body of the fertilised female swells and, as they die, it forms a tough, protective covering (the 'cyst') which contains the fertilised eggs (Whitehead, 1998). The sensory organs are composed of an inner and outer ring of papillae and a pair of amphid openings on either side of the mouth (Perry & Aumann, 1998). The amphidial exudates consist largely of sugars (Forrest & Robertson, 1986) and have a chemosensory role (Zuckerman, 1983).

### **A.1.7 Biology of potato cyst nematodes**

#### **A.1.7.1 Life cycle**

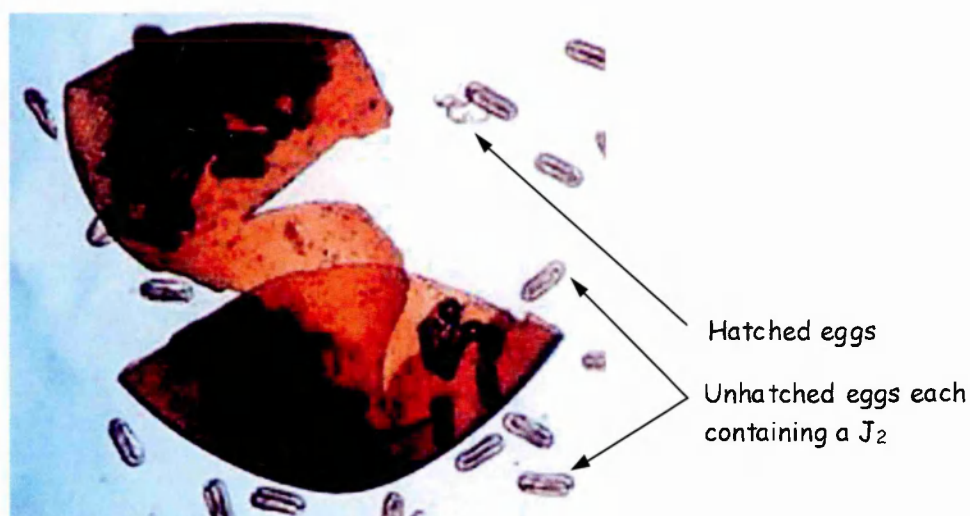
Potato cyst nematodes are monocyclic pests that reproduce sexually (den Ouden, 1960; Whitehead, 1998). A characteristic feature of their biology is the formation of the tough, leathery cyst (about 0.5-1 mm in diameter) from the body of the dead female (Franco, 1986). The cyst protects the eggs and the developing juveniles from desiccation, predation and chemical control (Jones *et al.*, 1998). These organisms are well-adapted root parasites that hatch only under favourable environmental conditions (suitable temperature, oxygen availability, appropriate soil moisture, absence of physiological barriers) in response to hatching chemicals leaching from host roots (Jones *et al.*, 1998). In the absence of host plants, cysts of *G. pallida* and *G. rostochiensis* can contain viable unhatched eggs for up to 20 years, after which their lipid reserves become depleted and they die (Turner, 1985).

Each PCN cyst may contain 100-600 eggs (Fig. A.3), with a single juvenile curled within each egg (Jones & Jones, 1984; Greco, 1988) (Fig. A.4, Stages 1 and

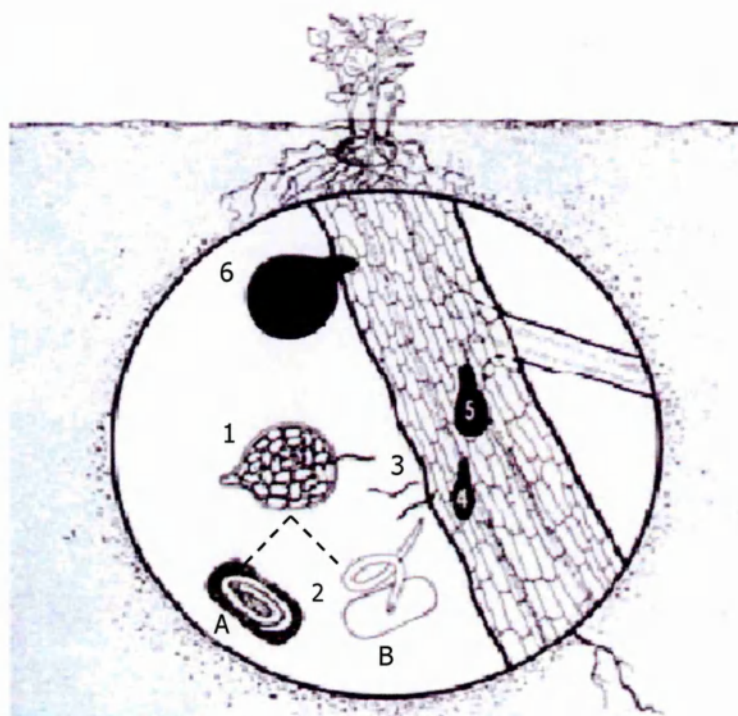
2A). Potato cyst nematodes have four juvenile stages and each of them is terminated by a moult (Franco, 1986). The first-stage juveniles (J<sub>1</sub>s) develop and moult within the egg. After the first moulting, under favourable environmental conditions, the J<sub>2</sub>s hatch from the eggs (Fig. A.4, Stage 2B) and emerge from the cyst (Jones *et al.*, 1998). A proportion of these J<sub>2</sub>s hatch spontaneously (i.e. in the absence of a suitable host; Tsutsumi, 1976), which could be the result of a microbial influence in the PCN hatching mechanism as later demonstrated by Devine *et al.* (1996).

The hatched J<sub>2</sub>s start moving between moist soil particles seeking the root, which they eventually locate by responding to various stimuli from the host root (Perry, 1997). For example, carbon-dioxide gradient established around the roots may help J<sub>2</sub>s to orientate towards the host (Prot, 1980; Perry, 1997). In *G. rostochiensis*, J<sub>2</sub>s need to locate the host roots as quickly as possible after they have hatched (Perry, 1997). In the absence of the host plant, J<sub>2</sub>s can only survive for 2 weeks in the field (Storey, 1984) and will normally lose their infectivity within 6-11 days from hatching (Robinson *et al.*, 1987) due to increased utilisation of their lipid reserves after this event.

Once they locate the root, the J<sub>2</sub>s invade it just behind the root tip or near the point of emergence of lateral roots (Trudgill *et al.*, 1996) (Fig. A.4, Stage 3). The J<sub>2</sub>s move within the root intercellularly through the cortical cells until they reach alongside vascular tissues where they initiate the formation of feeding sites. Once these sites have been established, J<sub>2</sub>s become sedentary with their heads near the endodermis and opposite a phloem tube (Jones & Jones, 1984) and they form giant feeding cells, called syncytia (Hooker, 1981) (Fig. A.4, Stages 4 and 5). The formation of syncytia, which, according to Rice *et al.* (1985), contain cells of the cortex, endodermis, pericycle and vascular parenchyma, is the result of the



**Fig. A.3.** Eggs released from a crushed PCN cyst  
(Credits: Department of Sustainability and Environment, Victoria, Australia)



**Fig. A.4.** Potato cyst nematode life cycle; 1) cyst in soil with enclosed eggs; 2) enlarged eggs showing a second-stage juvenile (J<sub>2</sub>): A. coiled inside the egg and B. hatching; 3) J<sub>2</sub>s entering the root; 4) and 5) developing female feeding on the root; 6) mature female emerging from the root (Hooker, 1981)

dissolution of root cell walls by salivary substances released by the J<sub>2</sub>s through their stylets (Melillo *et al.*, 1990). These substances are mainly cellulases produced by the subventral gland cells (Smart *et al.*, 1998). The J<sub>2</sub>s then become thicker and shorter and they moult to become third-stage juveniles (J<sub>3</sub>s). The body of the J<sub>3</sub> progressively enlarges rupturing the root cortex to which it is attached. The sex of the nematode is determined at the J<sub>3</sub> stage (Greco, 1988) by a number of factors, which include environmental conditions (Trudgill, 1967), host-parasite compatibility, competition for feeding sites (Mugniery & Fayet, 1984), and nutritional capacity of the syncytium; Jones & Jones (1984) estimated that the females need 500-1000 times more nutrients than the males. In heavy infestations with a limited food supply the population is predominantly male, whereas females predominate when the food supply is abundant and the population is low (Franco, 1986). The fourth stage males (J<sub>4</sub>s) re-elongate inside the J<sub>3</sub> cuticle and after the fourth moult they escape into the soil where they survive for up to 10 days (Evans, 1970). The female J<sub>4</sub>s continue to enlarge and to rupture the root cortex. At this stage, females have their heads buried inside the root, feeding, and the lower parts of their bodies exposed. The female J<sub>4</sub>s secrete pheromones (Green, 1980) which attract males, and fertilisation occurs outside the root. After the adult females containing the fertilised eggs have matured, they die and their body wall turns brown in both species due to the degradation of polyphenols by polyphenol oxidase in the cyst wall (Franco, 1986), thus creating the protective envelope described earlier known as the 'cyst' (Fig. A.4, Stage 6). The cysts may remain attached to the roots for up to 4 weeks (Ross, 1986) and they are usually shaken off the roots during harvesting (Ayers & Lane, 1986).

There is usually only one full generation per potato-growing season in *G. pallida* and *G. rostochiensis* (Whitehead, 1998), which, in western Europe, is

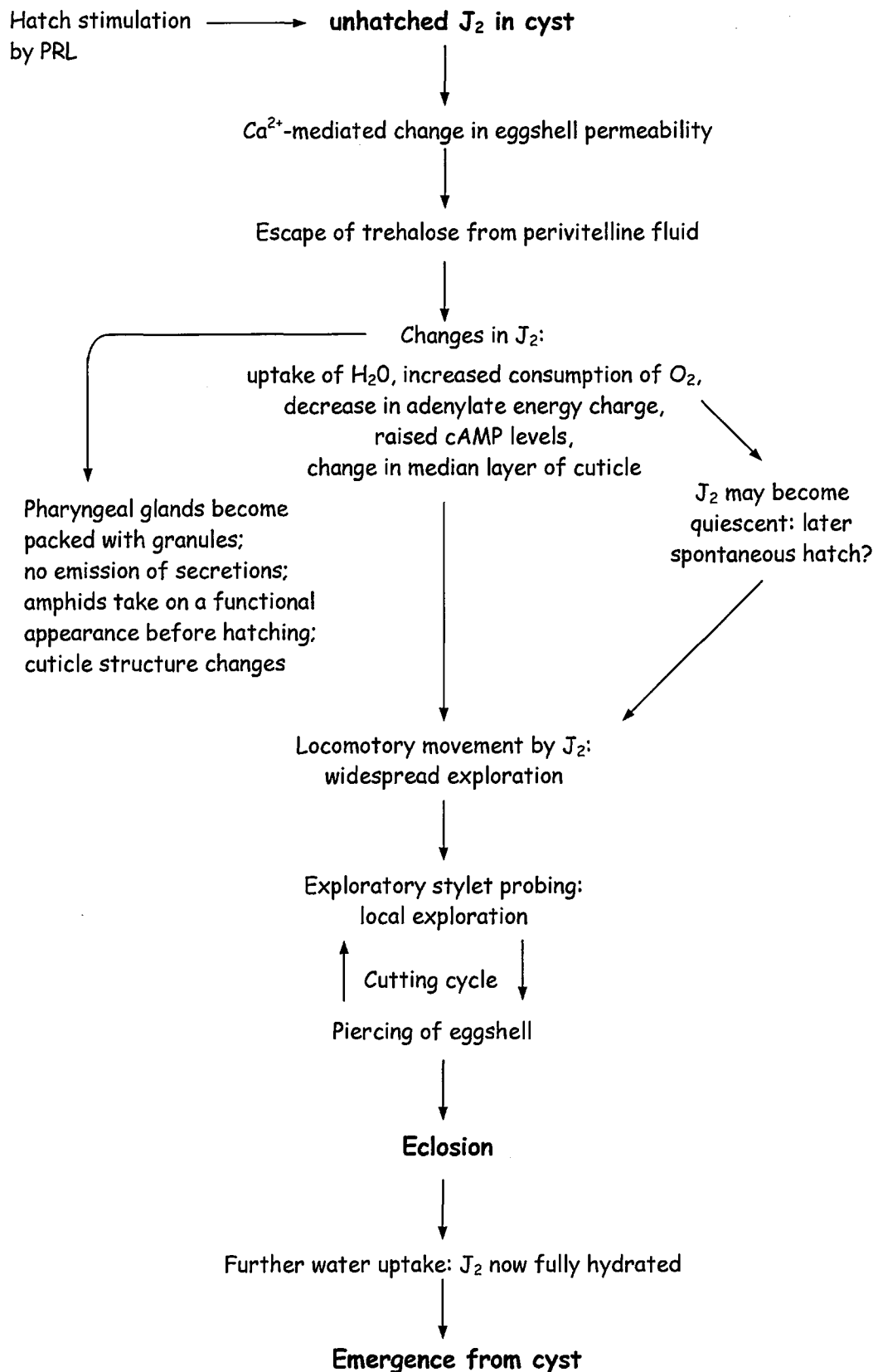
completed within 81-93 days (Webley & Jones, 1981), with the J<sub>2</sub>s hatching in April and the new generation appearing from late June (Ayers & Lane, 1986). Some J<sub>2</sub>s may hatch before the female becomes a cyst and, if soil moisture is adequate, they may invade the root and complete a second, but smaller, generation (Whitehead, 1993). Soil temperature is an important factor affecting the PCN life cycle, with *G. pallida* J<sub>2</sub>s being better adapted to hatching at lower temperatures than *G. rostochiensis* (Robinson *et al.*, 1987). Consequently, under such conditions, *G. pallida* may complete its life cycle faster than *G. rostochiensis* and hence, be more successful than *G. rostochiensis* in mixed PCN populations in the field, as has been demonstrated in the UK at soil temperatures of 13-14 °C (Lane & Holliday, 1974).

### **A.1.7.2 Hatching**

#### **A.1.7.2.1 The hatching process**

In comparison with *G. pallida*, the hatching mechanism of *G. rostochiensis* has been more extensively studied. A diagrammatic outline of the PCN hatching process is shown in Fig. A.5. While inside the egg, the J<sub>2</sub> of PCN is surrounded by perivitelline fluid which contains the sugar trehalose at a concentration of 0.34 M (Clarke *et al.*, 1978). This concentration of sugar keeps the juvenile partially dehydrated. The water content of J<sub>2</sub>s at this phase is 66% for *G. pallida* and 67% for *G. rostochiensis* (Clarke *et al.*, 1978), compared to 72% for fully hydrated J<sub>2</sub>s (Ellenby & Perry, 1976). The eggshell of PCN consists of three layers (external: lipoprotein; middle: chitinous; inner: lipid), of which the inner lipid layer is the main permeability barrier of the eggshell membrane (Wharton, 1980). While the eggshell allows sufficient supply of oxygen to the juvenile (Ellenby, 1968) it prevents entry of





**Fig. A.5.** Sequence of events in the hatching process of potato cyst nematodes after stimulation with potato root leachate (PRL) (from Jones *et al.*, 1998)

water into the egg, thus keeping the J<sub>2</sub>s in an immobile state (Clarke & Hennessy, 1976).

In the presence of potato root leachates (PRL), hatching factors (HFs) function by changing the permeability of the eggshell membrane from selectively-permeable to fully-permeable and this causes trehalose to diffuse from the egg (Jones *et al.*, 1998). The change in the membrane permeability is a calcium-mediated process (Clarke & Perry, 1985). Atkinson *et al.* (1980) and Clarke & Hennessy (1987) proposed that HFs bind to or displace the internal Ca<sup>2+</sup> of the membrane, leading to changes in the permeability of the innermost lipid layer and subsequently causing trehalose to leak from the egg. Devine *et al.* (1996) demonstrated that the potato steroidal glycoalkaloids  $\alpha$ -solanine and  $\alpha$ -chaconine stimulated the hatch of *G. rostochiensis*, and Jones *et al.* (1998) proposed that the aglycone was inserted into the membrane bilayer, thus causing a rearrangement of the membrane which disrupted the bilayer and caused subsequent leakage of trehalose. Perry & Feil (1986) reported that, in *G. rostochiensis*, the changes in the eggshell permeability occur within 12 h of exposure to PRL, although a prolonged exposure of at least 3 days is required for eclosion of J<sub>2</sub>s (Doncaster & Shepherd, 1967). It has been shown that exposure of eggs to PRL for just 5 min per week was enough to cause hatch stimulation (albeit sub-optimal) in both *G. pallida* (Perry & Beane, 1982) and *G. rostochiensis* (Forrest & Perry, 1980). The escape of trehalose through the eggshell reduces the osmotic pressure on the unhatched J<sub>2</sub>, thus allowing its water content and metabolic activity (e.g. oxygen uptake and energy consumption) to increase (Ellenby & Perry, 1976; Atkinson & Ballantyne, 1977; Jones *et al.*, 1998). These changes in the juvenile metabolism enable the J<sub>2</sub> to become more hydrated, active and, eventually, fully mobile within the egg (Jones *et al.*, 1998). Once hydrated, the J<sub>2</sub>

starts exploring the inner egg surface until it finds a site where local exploration occurs with the aid of the lips and the stylet. Only the head moves at this stage and the J<sub>2</sub> finally exits the egg from a slit in the eggshell made by repeated perforations of the membrane by the stylet (Doncaster & Seymour, 1973). Once hatched, the J<sub>2</sub>s (Fig. A.6) take up further water becoming fully hydrated after 2 days and they leave the cyst through the anal or vulval openings (Ellenby & Perry, 1976).



**Fig. A.6.** Hatched second-stage juveniles of the potato cyst nematode *Globodera rostochiensis*

#### **A.1.7.2.2 Hatching chemicals**

Hatching of J<sub>2</sub>s in both PCN species is greatly stimulated in the presence of PRL (Perry, 1989), which contains chemicals termed hatching factors (HFs; Devine *et al.*, 1996). Two further categories of hatching chemicals have been identified in PRL. Using picrolonic acid as an artificial HF, Byrne *et al.* (1998) found that some

of the chemicals present in PRL reduced the levels of hatching response of *G. rostochiensis* to this artificial HF, while others stimulated increased hatch in the presence of picrolonic acid but had no effect in isolation on hatching. They subsequently termed these chemicals as hatching inhibitors (HIs, chemicals which reversibly inhibit HF-induced activity) and hatching factor stimulants (HSs, chemicals which stimulate HF activity but alone are hatch-inactive), respectively.

#### A.1.7.2.3 Factors affecting hatch

Maximum production of HFs in PRL is achieved at or just behind the root tips (Rawsthorne & Brodie, 1986), which are also the points from which J<sub>2</sub>s invade the roots (Trudgill, 1986). Jones *et al.* (1998) reported that coarse-texture soils favour hatching and subsequent root invasion, providing that aeration is sufficient and conditions for J<sub>2</sub>s migration are suitable. In the field, the response of the two PCN species to HFs depends on the availability and mobility of these factors in the soil solution as demonstrated by Devine *et al.* (2001). These authors found that HFs exhibiting *G. pallida*-selective and -specific hatching activity showed less affinity for the soil matrix than did those for *G. rostochiensis* and also that the selectivity of the earliest eluting HFs towards *G. pallida*, relative to *G. rostochiensis*, increased significantly as the percentage soil organic matter content increased. Soil temperature is another important factor affecting hatch in PCN (Section A.1.7.1).

Direct evidence has been published confirming that hatching of J<sub>2</sub>s is synchronised with the growth of the potato plant. Twomey (1995) found that production of hatch-active leachate coincided with shoot emergence. Byrne (1997) found that potatoes started to produce hatch-active leachate even before shoot emergence and that maximum hatching activity in the PRL occurred within 3 weeks

after this event. Byrne (1997) also demonstrated that the composition of HF's in the PRL varied with the growth stage of the potato plants. Trudgill *et al.* (1996) reported that hatching of *Globodera* spp. in the field occurs over an 8-week period, with *G. pallida* generally exhibiting a slower hatch rate than *G. rostochiensis*, while LaMondia & Brodie (1986) found that the majority of *G. rostochiensis* J<sub>2</sub>s which successfully invaded the potato roots hatched within the first 3 weeks. Byrne *et al.* (2001) described apparent preferences for HF's leaching from potato roots to the two PCN species, although both hatched in response to each HF; these HF's are described as species-selective HF's. On the other hand, species-specific HF's would induce hatch of only one PCN species; the other species is not affected.

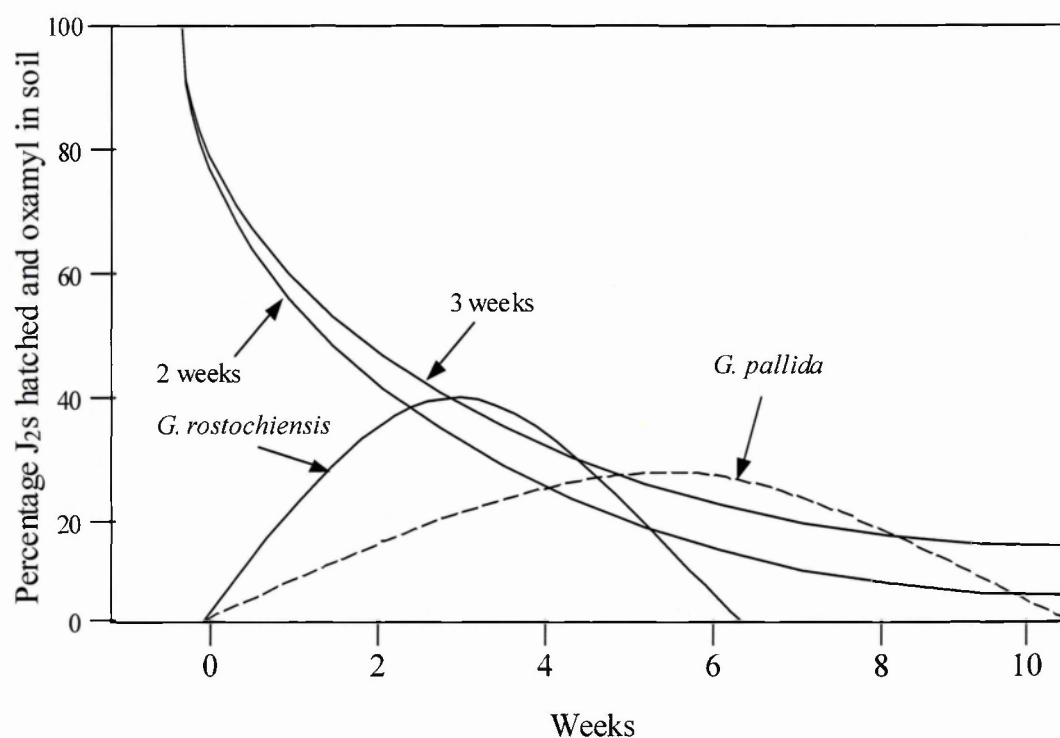
Jones *et al.* (1998) suggested that application of HF's isolated from PRL in soil infested with PCN, either fallow or planted to a non-host, could lead the hatched J<sub>2</sub>s to starvation and subsequent death because of the absence of host roots, a phenomenon described as 'suicide hatch'; this was later confirmed by Devine & Jones (2000). These authors reported that the hatching response of PCN (species used *G. rostochiensis*) was found to be PRL and tomato root leachate (TRL) concentration dependent and concluded that the response of PCN to natural, exogenously applied, HF's in soil involves both hatch and in-egg mortality. The main principles on which the 'suicide hatch' strategy has been based are the almost complete dependence of PCN on host root leachate (PRL or TRL), containing multiple HF's (Devine *et al.*, 1996) to break quiescence and permit hatch (Devine & Jones, 2000) and the limited viability of hatched J<sub>2</sub>s in the absence of a host plant (Storey, 1984; Robinson *et al.*, 1987; Devine, 2000) (Section A.1.7.1). Interest in the development of PCN 'suicide hatch' control strategy was developed after experimental evidence was published for the persistence (Whitehead, 1973;

Tsutsumi, 1976) (Section A.1.10.8) and high mobility (Rawsthorne & Brodie, 1987; Jones *et al.*, 1998; Devine *et al.*, 2001) (Section A.1.7.2.3) of HF in soil. The successive stages of the 'suicide hatch' method are the following: (i) HF preparation, (ii) HF application to PCN-infested soil, (iii) monitoring of the effect, and (iv) recording of the findings; details of each of this stage as well as other important parameters that must be considered when following this approach (e.g. timing of HF applications to the field) can be found in Devine (2000) and Devine & Jones (2000). Some selected results of recent 'suicide hatch' experiments are presented in Section A.1.10.8.

#### **A.1.7.3 Differences in the biology of the two potato cyst nematode species**

The need to find alternative, more environmentally friendly methods to control PCN combined with the poor effectiveness of the currently available nematicides against *G. pallida* has resulted in the accumulation of a considerable amount of information on the biology of the two PCN species. Particular emphasis has been given to a better understanding of the PCN hatching mechanism. In the UK, the various control methods employed to control PCN have resulted in important changes in the field populations of the two PCN species (Section A.1.2) and this has been partly the result of differences in the biology of the two PCN species (Evans, 1993; Haydock & Evans, 1998). The inadequate control of *G. pallida* population increase (relative to *G. rostochiensis*) by the short-persistence granular nematicides such as aldicarb and oxamyl (Whitehead, 1992; Ambrose *et al.*, 2000) has been associated with the slower rate of emergence of *G. pallida* J<sub>2</sub>s, compared to those of *G. rostochiensis* (Fig. A.7). According to Evans (1983), this is probably due to differences in the times required by the two PCN species to absorb water and hence,

to respond to host root leachates. Den Nijs & Lock (1992) reported that *G. pallida* appears to be more dependent than *G. rostochiensis* on the presence of PRL to trigger hatch. The most important differences in the biology of the two PCN species are summarised in Table A.2.

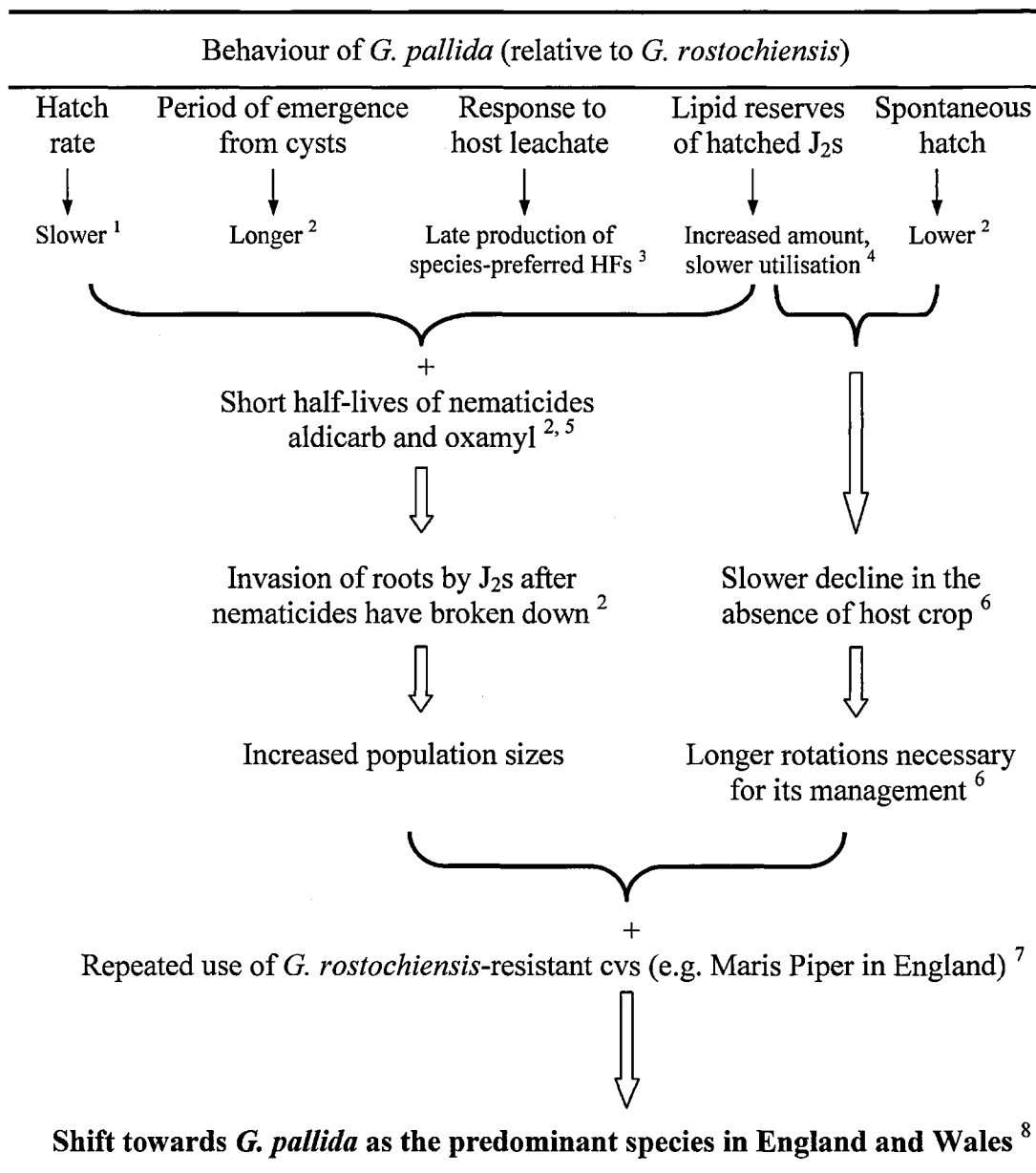


**Fig. A.7.** Comparative hatching behaviour of *G. pallida* and *G. rostochiensis* J<sub>2</sub>s under a potato crop and decay curves for oxamyl with 2 or 3 weeks half-lives (from Haydock & Evans, 1998)

#### A.1.7.4 Arrested development, diapause and quiescence

Under special circumstances PCN enter a period of arrested development known as dormancy. Dormancy can occur at any stage of the PCN life cycle and it is an adaptive mechanism employed by the nematodes in order to overcome discontinuous unfavourable environments (Maggenti, 1971; Jones *et al.*, 1998). It enables both species of PCN to persist in the soil for long periods that may exceed 20 years and then to hatch within days of detecting host root leachates in the soil solution (Jones *et al.*, 1998). The two types of dormancy exhibited by PCN are

**Table A.2. Differences in the biology and in-soil behaviour of the two potato cyst nematode species and implication on control tactics**



<sup>1</sup> Trudgill *et al.*, 1996, <sup>2</sup> Whitehead, 1992, <sup>3</sup> Byrne *et al.*, 2001, <sup>4</sup> Robinson *et al.*, 1985, <sup>5</sup> Ambrose *et al.*, 2000, <sup>6</sup> Marshall, 1984; Haydock & Evans, 1998; Whitehead & Turner, 1998, <sup>7</sup> Urwin *et al.*, 2000, <sup>8</sup> Minnis *et al.*, 2002.

diapause and quiescence (Antoniou, 1989). Diapause is a type of arrested development which enables nematodes to overcome seasonal, long-term conditions which are unfavourable for hatch and root invasion, such as extreme winter or summer weather and/or absence of host plants (Jones *et al.*, 1998). Diapause does not



terminate until specific requirements have been satisfied, even under favourable environmental cues (Williams, 1978; Jones *et al.*, 1998); Antoniou (1989) reported that any attempt to force J<sub>2</sub>s to hatch while they were in diapause, resulted in either their death or in loss of virulence. Hominick *et al.* (1985) described diapause as a survival strategy employed by the nematode in order to synchronise its life cycle with that of the host plant.

Diapause can be distinguished in obligate and facultative forms. In *G. rostochiensis* in western Europe, the newly-formed unhatched J<sub>2</sub>s entered a period of obligate diapause, which occurs from autumn to spring in the first year after the eggs are formed ('first season diapause'; Ellenby & Smith, 1967). Obligate diapause in *G. rostochiensis*, which can occur only once during its life cycle (Hominick, 1986), is initiated by signals from the host plant to the developing J<sub>2</sub>s via the maturing female (Hominick *et al.*, 1985). Factors affecting the length of obligate diapause include photoperiod (negative correlation; Hominick, 1986) and host genotype (Evans, 1982). Temperature is the most important environmental cue for the breaking of obligate diapause, with unhatched J<sub>2</sub>s requiring a period of exposure to low temperatures before exiting obligate diapause (Antoniou, 1989). In contrast with obligate diapause, facultative diapause is initiated by exogenous stimuli (predictable environmental changes; Jones *et al.*, 1998), which, in *G. rostochiensis*, include daylength (Franco & Evans, 1979) and low temperatures (Hominick, 1979). Facultative diapause in PCN occurs from the second season onwards (Jones *et al.*, 1998) and is terminated in the spring when the soil temperature increases (Antoniou, 1989).

Quiescence is a type of arrested development which PCN enters spontaneously as a reaction to unexpected unfavourable environmental conditions (Antoniou, 1989).

These conditions, which can occur either singly or in combination, include lack of water (anhydrobiosis) during dry summer periods (Evans & Perry, 1976), osmotic stress (osmobiosis) due to high salt concentrations in the soil (Clarke *et al.*, 1978), lack of oxygen following flooding or high rainfalls (Sayre & Hwang, 1975), extremely low (cryobiosis; Oydvin & Hammeraas, 1973) or high temperatures (thermobiosis; Perry & Wharton, 1985), or absence of the host plant (Jones *et al.*, 1998). Quiescence can occur at any time of the year, but is readily reversible when favourable conditions return (Antoniou, 1989). Two types of quiescence have been described, obligate and facultative (Evans & Perry, 1976). The obligate quiescence occurs in temperate regions in early spring, when a specific receptive stage of the nematode life cycle is affected by the extreme environment (e.g. quiescence of unhatched J<sub>2</sub>s in the absence of PRL; Jones *et al.*, 1998). It is then terminated by an increase in soil temperature and by the presence of PRL, which will stimulate PCN to hatch. On the contrary, facultative quiescence may occur irrespective of the stage in the PCN life cycle (Jones *et al.*, 1998). In comparison with *G. rostochiensis* J<sub>2</sub>s, *G. pallida* J<sub>2</sub>s require greater amounts of PRL to exit quiescence (den Nijs & Lock, 1992).

### **A.1.8 Detection of potato cyst nematodes in the field and studies of population dynamics**

#### **A.1.8.1 Soil sampling and processing**

Soil sampling is necessary to determine whether PCN is present or absent from the field where the potato crop will be planted. In ware potato production, the first sampling is normally conducted the autumn before planting to assess the initial PCN population size ( $P_i$ ) and thus to determine the risk to the crop to be planted. Soil

samples are also taken, in experimental situations, after the crop is lifted to assess the final PCN population size ( $P_f$ ) and hence, the multiplication rate ( $P_f/P_i$ ) on that crop. Post-harvest sampling also provides an indication of likely damage to the next crop (Seinhorst, 1992). Monitoring of the PCN population densities in the field is an essential part of integrated PCN control systems (Hockland *et al.*, 2000) and it also determines whether the PCN management procedures are adequate for the prevention of unacceptable yield losses (Haydock & Evans, 1998). In seed potato production areas, fields are sampled with the purpose of ensuring that they are free of PCN (Hockland *et al.*, 2000). For research purposes, samples of soil may also be taken during the growing season to assess PCN hatch levels (associated with granular nematicide efficacy) or during crop rotation to determine the PCN decline rate.

The PCN field population dynamics are determined by the age of infestation and the frequency of potato cropping since introduction (Haydock & Evans, 1998). The distribution of the PCN population within the field can be very patchy, especially at low population densities (Riding & Parker, 2000), making the detection of initial PCN foci very difficult. Therefore, for a sampling strategy to be reliable, it should include as many sampling points as possible. Problems associated with the intensive field sampling for PCN detection include the high costs involved and the length of time required for processing the samples (Haydock & Evans, 1998). Recent advances towards the development of more reliable sampling regimes are the use of the Global Positioning System (GPS) and Geographical Information Systems (GIS) (Riding & Parker, 2000). These technologies produce maps of PCN infestations within a field and therefore are a very useful tool in improving the result of the nematicide application and in reducing the associated costs.

The accuracy of the estimation of the PCN population size within a field depends on the way the soil is sampled (Shepherd, 1986). The sampling and extraction systems followed currently by the Plant Health Service in the UK are based on the European and Mediterranean Plant Protection Organisation (EPPO) Quarantine Procedure for *G. pallida* and *G. rostochiensis* (Anon., 1991). In practice, however, there is a broad variety of sampling schemes (e.g. variation in the sample size, location and depth of sampling). Factors such as the size of the area to be sampled, the soil type and the PCN population density in the previous crop influence selection of the sampling technique.

Samples are usually taken to plough depth (Shepherd, 1986; Turner, 1993). Although PCN cysts can be found very deep in the soil, the majority of them are limited to the top 20 to top 50 cm of the soil profile (Whitehead, 1977). The depth distribution of PCN cysts in the soil is affected by various parameters such as ability of roots to penetrate the soil profile, cultivar susceptibility, previous cropping history and ploughing depth. There is no general rule regarding sampling depth for soil collection; Bakker *et al.* (1978) suggested that soil samples must be collected from the top 20 cm. A typical corer used by nematologists to collect soil samples is the 'cheese-corer' (20 cm x 2.5 cm diameter) style auger with a half-cylindrical blade (Minnis *et al.*, 2002). Soil samples are usually taken in systematic distribution which may be a grid pattern or a 'w'-shaped path (Hockland, 2002). Each sample is derived from sufficient points within the field to ensure that it is representative of the area sampled. For example, using the 20 cm x 2.5 cm corer, a 2 kg bulked soil sample can be made up of 50 separate soil cores.

The bulked soil sample is usually collected in cotton or linen bags and it is then air-dried in ventilated cupboards equipped with tubular heaters and a fan to ensure

circulation of warm air around the samples (Shepherd, 1986). The following step is to crush the soil and pass it through a 4 mm sieve to remove any stones and coarse gravel present (Shepherd, 1986). Usually only one subsample is taken from the total sample after the soil is thoroughly mixed. The size of the sample depends on the soil type, the extraction method and nematode density (McSorley, 1987). The sample size is determined by weight or volume and usually subsamples of 100, 200 or 500 g are taken for cyst extraction (Shepherd, 1986).

#### **A.1.8.2 Extraction of cysts**

Several floating techniques have been developed to extract cysts from soil samples and they are all based on the characteristic that dry cysts float in water. The most common method of cyst extraction is the Fenwick can (Fenwick, 1940). This apparatus is suitable for moist or air-dried soil samples up to 300 g, and recovers about 70% of the cysts in the sample (Shepherd, 1986). The cysts and the organic debris are eluted on a 250 µm sieve. To obtain a cleaner sample, cysts can be separated further by a brief elutriation in a glass cylinder filled with water (Shepherd, 1986).

Since the construction of the Fenwick can, several other techniques have been employed for separating cysts from soil samples. Trudgill *et al.* (1972) constructed a fluidising column, which was suitable for extraction of any types of nematodes, including PCN cysts, from 50-100 g of soil. However, the principal value of this fluidising column is for separating mature and immature females of PCN from root washings and soil (Shepherd, 1986). Using this column, cysts and organic debris are separated in an upward current of water. Winfield *et al.* (1987) constructed a fluidising column suitable for separating cysts from larger samples of moist soil (up

to 200 g). The methods of cyst extraction using elutriators can be used for wet soil and are based on the principle that cysts have different densities than the soil particles (Turner, 1998).

Semi-automatic flotation methods for separating cysts from soil have also been developed, such as the 'Schuiling Centrifuge' manufactured by the Netherlands Inspection Service for Field Seeds and Seed Potatoes (NAK). In comparison with the Fenwick method, this model gives a cleaner residue for cyst extraction but it has proven unsatisfactory for soils with a high organic matter (Shepherd, 1986).

Cysts of *Globodera* spp. are usually left to dry before being recovered from the final organic debris. A widely used method of cyst separation from air-dried floats as well as wet floats is that of Winfield *et al.* (1987), where cysts and organic matter are washed onto a filter paper in a separate funnel and the cysts are subsequently recovered from a ring that is created around the top of the filter paper. There are several other methods for removing the cysts from the debris, such as by rolling or sieving. Details of each of these methods are described by Southey (1970), Shepherd (1986) and Turner (1998). After the cysts have been recovered they can be counted under a stereomicroscope using the counting tray of Fenwick (1940).

#### **A.1.8.3 Estimation of cyst contents**

Due to the large variation in egg content exhibited by PCN cysts, nematologists usually express the result of soil sampling for PCN infestation as eggs g<sup>-1</sup> dried soil or as eggs ml<sup>-1</sup> soil, rather than as cysts per weight or per volume of soil. Presentation of eggs cyst<sup>-1</sup> is often included in the results as it provides useful information on the effects of the various control tactics on the PCN life cycle.

As with cyst extraction, several methods are in use for liberating and determining the cyst contents (eggs and juveniles). Cysts can be opened with a dissecting needle or an oculist's scalpel, but when the sample to be analysed is large, these methods are time consuming and unreliable because they determine the number of eggs only in a proportion of the total cyst population (Shepherd, 1986). To overcome this problem, many nematology laboratories nowadays use the channelled aluminium slide (7.5 x 2.5 cm) on which the cysts are crushed with the aid of a glass rod. The crushed sample is then washed off the slide into graduated cylinders and eggs and juveniles are subsequently counted in aliquot samples using any of the several available counting slides, such as Fenwick's multichamber counting slide (Fenwick & Franklin, 1951). Counting is done under a stereomicroscope (x50 magnification) with the aid of a hand tally counter.

#### **A.1.8.4 Hatching tests and viability estimation**

Various methods have been developed for determining hatch levels in PCN. A standard procedure, which is still widely used, is that of Fenwick & Widdowson (1958). Using this technique, batches of cysts (usually 100 per replicate) are placed in a watch glass, and the number of J<sub>2</sub>s emerging from the cysts into sample aliquots is determined at weekly intervals. This hatching test procedure is time-consuming, in terms of both the duration of the experiment (at least 3 weeks) and the time required to complete the hatch counts (Jones & Gander, 1962).

The microtitre plate bioassay method of Twomey *et al.* (1995) is an improved procedure for assaying the hatching activity of PRL towards the two PCN species. The advantages of this design compared to that of Fenwick & Widdowson (1958) are the reduced time required to count the hatched J<sub>2</sub>s, the reduction of error in estimates

of hatching activity (cysts are graded into three size classes: < 300 µm, 300-500 µm and > 500 µm; this reduces the within-cyst variability in egg content and hatching rate) and the smaller amounts of HF sample needed.

A problem nematologists encounter when quantifying PCN population sizes in the soil is deciding when eggs and juvenile nematodes are dead. When investigating the effectiveness of a PCN control method, only the viable, potentially infective, eggs of the population are of interest. Although many different methods have been developed for measuring viability of cyst nematodes, none of them is entirely satisfactory (Shepherd, 1986). For example, failure of J<sub>2</sub>s to hatch in simple hatching tests does not necessarily signify death, as eggs may be in a dormant stage, so that direct observation will not distinguish between viable and dead eggs (Shepherd, 1962). To overcome this problem, various dyes have been tested to differentiate between living and dead nematodes (Table A.3). From these stains, probably the most widely used is the New blue R (Meldola Blue) of Shepherd (1962). When using this stain, cysts of *Globodera* spp. are soaked for 1 week in water and are then immersed for a further week in 0.05% (w/v) aqueous solution of the stain. Following this, the stain is removed, the cysts are soaked in water overnight to remove excess unbound stain and the eggs are released by crushing the cysts. The New blue R stain appears to enter the body of dead nematodes through oral and anal openings first, which are consequently stained blue, deep purple or almost black. On the contrary, in living juveniles, neither the cuticle nor the body contents take up the stain (Shepherd, 1962).



**Table A.3. Stains used to differentiate between living and dead nematodes**

Stain	Reference
1) Iodine in potassium iodide	Boyde (1941)
2) Acridine orange	Homeyer (1953)
3) Chrysoidin	Doliwa (1956)
4) Phloxine B	Fenner (1962)
5) New blue R (Meldola blue)	Shepherd (1962)
6) Nile blue A	Ogiga & Estey (1975)

### **A.1.9 Symptoms and damage caused by potato cyst nematodes**

The damage caused by PCN in potato plants is initiated when invading J<sub>2</sub>s migrate through the roots, destroying root cell walls and eventually forming syncytia (Section A.1.7.1). However, the damage becomes visible only at certain infestation levels, depending on conditions such as soil fertility and water supply (Franco, 1986). At the earlier stages of invasion, the damage is often inconspicuous (Franco, 1986). The roots are substantially damaged and symptoms appear on the plant only when large numbers of juvenile nematodes feed simultaneously on the roots (Haydock & Evans, 1998). Plants from cultivars with low tolerance to PCN attack are damaged more severely than plants with high PCN tolerance (Evans & Haydock, 1990).

As PCN invasion of host plant roots progresses, uptake of water and nutrients such as nitrogen (N), phosphorus (P) and potassium (K) is reduced (Trudgill *et al.*, 1975; Grove *et al.*, 1999). Heavily infested plants have smaller root systems, which are abnormally branched and brownish in colour (Whitehead, 1998). Affected plants exhibit various symptoms of water and nutrient deficiency, such as stunted growth, wilting, chlorotic leaves and reduced canopy development and eventually, they may die or senesce prematurely (Haydock & Evans, 1998), although in some situations

this could also be the result of increased susceptibility to *Verticillium* wilt disease associated with PCN feeding damage (Whitehead, 1998). Grove *et al.* (1999) reported that the reduced canopy in affected plants affects the amount of solar radiation intercepted by the leaves, resulting in a reduction of dry matter production and hence, of tuber yield. However, the same authors demonstrated that a foliar N fertiliser increased the yield of PCN-infested plants (cvs Pentland Dell and Santé) but only when the recommended quantity of N for the crop was split between planting and tuber initiation. At extremely high PCN population densities, both the size and number of tubers are reduced, and the tubers may become infected, resulting in the appearance of females and cysts on their surface mid-season (Whitehead, 1998). Secondary pathogens often invade the roots at the points of invasion by PCN (Trudgill *et al.*, 1987). Corbett & Hide (1971) reported extensive damage in potatoes caused by *Verticillium dahliae* in soil infested with large numbers of *G. rostochiensis*.

While the symptoms of PCN infestation in individual plants are not specific, as other causes may lead to similar symptoms, in the field as a whole the appearance of patches of poor growth is a good indication of the presence of damaging PCN levels. These patches of diseased plants (Fig. A.8) enlarge with each new crop if potatoes are continually cultivated on the infested site (Franco, 1986).

Losses from PCN infestation can be either direct due to yield reduction or indirect due to the expenses involved in controlling PCN increases in the field (Franco, 1986). In light infestations, potato plants may show no above ground symptoms, but yield losses can still be substantial. The level of yield reduction is related to the initial number of viable eggs  $\text{g}^{-1}$  soil ( $P_i$ ). A widely used model, which

relates total plant yield with log nematode density, is described by the sigmoid curve equation of Seinhorst (1965) that states:

$$Y = Y_{\min} + (1 - Y_{\min})cz^{P_i}$$

where  $Y$  = yield as a fraction of maximum yield (1.0),  $Y_{\min}$  = minimum yield as a fraction of maximum yield,  $P_i$  = number of nematodes at planting,  $c$  = a constant (1.05-1.15), representing 5-15% compensation by the plant for nematode injury and  $z$  = a constant (usually  $c. 0.995$ ), representing the fraction of maximum yield left after one nematode has fed (Whitehead, 1998). Equations based on linear regressions of tuber yield on eggs  $g^{-1}$  soil are also available (Brown, 1969). Trudgill (1986) concluded that the models of Seinhorst (1965) and Brown (1969) cannot be satisfactory for all sites. This could be due to the fact that important parameters affecting yield loss were not included in these model equations (Table A.4; Whitehead, 1998).



**Fig. A.8.** Patches of stunted plants in a potato field infested with potato cyst nematodes (Credits: H. P. Beukema, Potato Explorer-Netherlands Potato Consultative Institute, NIVAP)

**Table A.4. (a) Factors affecting yield losses in potato crops due to potato cyst nematode (PCN) damage (adapted from Whitehead, 1998) and (b) Effects of various PCN population densities (eggs g<sup>-1</sup> soil) on crop losses (adapted from Jones, 1973)**

(a)	➤	Initial nematode population density		
	➤	Degree of tolerance of cultivar grown		
	➤	Soil moisture stress		
	➤	Virulence of the nematode population		
	➤	Presence of interacting pathogens (e.g. <i>Verticillium dahliae</i> )		
(b)	Preplanting density ( $P_i$ )	Postharvest density ( $P_f$ )	Multiplication rate ( $P_f/P_i$ )	Crop loss (approx.)
	1	30	30	none
	10	100	10	little
	50	200	4	quarter
	100	300	3	half
	200	250	1.25	failure
	300	210	0.7	most

The economic threshold for crop loss due to PCN damage is usually less than 20 eggs g<sup>-1</sup> soil (Haydock & Evans, 1998), while the damage threshold is just 1.5-1.7 eggs g<sup>-1</sup> soil (Greco *et al.*, 1982). It has been estimated, based on linear regressions of tuber yield on eggs g<sup>-1</sup> soil, that when PCN infestation increases by 20 eggs g<sup>-1</sup> soil, tuber yield decreases by at least 2 tons per hectare (Franco, 1986; Whitehead, 1998). Evans & Brodie (1980) reported that the annual losses in potato production in the UK due to PCN damage were about 9% but a recent published assessment has not been done (Hockland, 2002). According to Haydock & Evans (1998) the corresponding losses at market value in the UK were approximately £43m based on the mean value of the crop from 1990-1995.

### **A.1.10 Management of potato cyst nematodes**

The first strategy for PCN management is the prevention of infestation of clean areas. If this is not possible various control tactics can be employed but these must be accompanied by the regular monitoring of the PCN population density and species composition in the contaminated field (Table A.5; Haydock & Evans, 1998). There are two targets in any control measure against PCN; reduced yield losses and reduction of the nematode population (Whitehead, 1986). Jones & Jones (1984) reported that, as PCN can multiply up to 70-fold in one season, at least a 99% reduction in the PCN population size is required for an effective control of PCN. However, at a very low  $P_i$ , at or below the tolerance threshold for the crop, the  $P_f/P_i$  may be as much as 150-fold, whereas at a very high  $P_i$ , the  $P_f/P_i$  may be as low as 1-fold or even below unity (Whitehead, 1998), due to the competition between juveniles for establishment of feeding sites in the roots, which results in the population becoming predominantly male (Phillips, 1984).

#### **A.1.10.1 Quarantine and legislation**

Potato cyst nematodes are easily introduced and spread to uncontaminated areas both locally and between countries (Section A.1.3) and, in many cases, this is combined with an uncontrollable increase in the PCN population levels (Section A.1.9). Consequently, unless effective control methods are applied within each country as well as internationally, PCN will continue to pose a risk to the potato production throughout the world (Hockland, 2002).

Organisations such as the Food and Agriculture Organisation (FAO), the International Plant Protection Convention (IPPC) and the European and Mediterranean Plant Protection Organisation (EPPO) have published lists with the

**Table A.5. The available strategies for the management of potato cyst nematodes (PCN) (adapted from Haydock & Evans, 1998)**

<u>Objectives</u>	<u>Methods</u>
✓ Prevention of infestation	Quarantine and legislation Farm hygiene
✓ Monitoring of population size	Soil sampling, cyst extraction and quantification
✓ Species monitoring	Biochemical or morphological/morphometrical methods
✓ Field management	Crop rotation Resistant cultivars Chemical control Trap cropping Integrated PCN management
<i>AIMS:</i>	
1) to reduce the PCN population size below the damage threshold for crop loss (short-term aim)	} INCREASE IN YIELD
2) to prevent multiplication of PCN in the years following (long-term aim)	

pests and pathogens (including PCN) that exist in each country and the measures needed to control them (Taylor, 1986). Moreover, the EC Council Directive 69/465/EEC (Anon., 1969) listed a set of rules to protect potato cultivation (seed production areas in particular) from PCN spread (Hockland *et al.*, 2000). Following this, most EU countries adopted quarantine legislation against both *G. pallida* and *G. rostochiensis* in an effort to prevent the spread of these pests to unaffected land (Taylor, 1986). The basic requirements of the PCN statutory management can be summarised as follows (Hockland *et al.*, 2000):

- Soil sampling should be conducted before planting seed tubers to ensure that the land is PCN-free.

- If seed potatoes are found contaminated by PCN, they should be decontaminated by thorough washing to remove all traces of soil and cysts.
- Contaminated farm machinery must be cleaned to avoid PCN spread to clean land.
- Seed potatoes for marketing may be grown only on land officially recognised as PCN-free.
- If the land is infested with PCN it should be demarcated ('scheduled') and:
  - no potatoes for seed production may be grown on it.
  - no plants intended for transplanting may be grown or stored either in this land.
  - only ware potatoes may be grown provided that: a) a PCN-resistant cultivar is used or b) the crop is harvested before PCN cysts mature (usually considered to be prior to 30 June).
- Seed potatoes for planting imported from a non-EU Member State must be accompanied by an official statement that they originate from a PCN-free field (i.e. phytosanitary certificate).

#### **A.1.10.2 Crop rotation**

Crop rotation was the only method followed by potato growers to control PCN populations until the 1960s (Evans & Haydock, 2000). Control of PCN by crop rotation is based on the principle that in the absence of the host potato crop the PCN population density progressively decreases due to spontaneous hatching of a proportion of J<sub>2</sub>s in the spring and/or to destruction of a proportion of eggs due to predation and parasitism (Whitehead, 1998; Elliott *et al.*, 2000). The continuous

cultivation of potatoes on infested land results in heavy infestations and substantial yield losses (Whitehead, 1998).

Important considerations in any crop rotation programme are the PCN population size and its annual decline rate; these parameters determine the number of non-host crops that must be grown to prevent injury to the next potato crop (Whitehead, 1998). Heavy soil infestations combined with slow PCN decline rates require very lengthy rotations (Table A.6), but any rotations exceeding 10 years are usually unacceptable commercially (Whitehead, 1998). The annual PCN decline rate in the absence of the host crop has been estimated at about 20% (Cole & Howard, 1962), 33% (Cooper, 1953) and 49% (den Ouden, 1974), while for modelling purposes the annual decline rate is assumed to be 30% (Hancock, 1988). Whitehead (1995) found that under continuous barley cropping, the average annual decline rate of *G. pallida* populations was 20%. Potato cyst nematodes decline rates vary greatly with the environmental conditions. In particular, the annual decline rate appears to be faster in the warm soils of tropical climates than in the cold soils of the temperate climates of north western Europe (Greco, 1993). Soil type may also influence the speed of PCN decline in a rotation programme (Whitehead, 1998). Greco (1988) reported that the decline rate of *G. rostochiensis* was slower in peaty soils than in other soil types. Evans & Haydock (2000) suggested that the variation exhibited between individual field populations of PCN in decline rates may be the result of genetic variability between populations, but also due to the different physical environments of different fields.

Hancock (1988) suggested a 7-8 year rotation for potatoes in order to achieve a satisfactory decline in PCN population. In the UK, however, the slower rate by which *G. pallida* declines in the soil, compared to *G. rostochiensis* (due to lower



**Table A.6. Years rest from solanaceous crops for decline of potato cyst nematode to 5 eggs g<sup>-1</sup> soil (Whitehead, 1998)**

Annual decline (%)	Eggs g <sup>-1</sup> soil		
	50	200	800
50	3.3	5.3	7.3
30	6.5	10.3	14.2
20	10.3	16.5	22.7

spontaneous hatch; Whitehead & Turner, 1998), means that rotations of such length may be insufficient to control *G. pallida*. Evans & Haydock (2000) reported that rotations as long as 8 years or more may be necessary to suppress some *G. pallida* populations even if nematicides are used. The survey of Minnis *et al.* (2002) showed that the current average rotation length followed by potato growers in England and Wales is 5.7 years. Usually the PCN decline during crop rotation is faster in the first year after production of eggs, as demonstrated by Turner (1996) who found that PCN populations declined by 73% over a 13-year period, with the highest rate of decline (*c.* 50%) being recorded in the first year.

#### **A.1.10.3 Resistant cultivars**

While all potato cultivars stimulate PCN hatch, some do so without providing subsequent sufficient food supply (Franco, 1986). These cultivars are PCN-resistant and by preventing the multiplication of invading nematodes they may reduce PCN infestation levels by 80% in a season and by 99% after 3 consecutive years of growing (Whitehead, 1998).

The first report of resistance to PCN involved cultivated lines of *Solanum tuberosum andigena* (tetraploid) and wild *S. vernei* (diploid) (Ellenby, 1954). Breeding of *S. tuberosum andigena* x *S. tuberosum tuberosum* gave hybrids resistant

to *G. rostochiensis*. These hybrids have a single, dominant gene ( $H_1$ ) resistant to pathotype Ro1/Ro4 (= Ro1). While resistance to *G. rostochiensis* is a major gene resistance and is usually complete (i.e. it allows no nematode reproduction), resistance to *G. pallida* is only partial and polygenetic (Whitehead, 1998). The National Institute of Agricultural Botany (NIAB) in Britain gave an assessment of the partial resistance to *G. pallida* that states: "The number of varieties with such (*G. pallida*) resistance is still relatively small. Partial resistance is estimated, from tests carried out in pots, by the amount of cyst multiplication in comparison to fully susceptible controls. Varieties giving a multiplication rate of less than 50% of the controls are classified as partially resistant" (Anon., 1996).

The most widely grown potato cultivar in the UK containing the  $H_1$  gene is cv. Maris Piper (Urwin *et al.*, 2000). Two other popular  $H_1$  cultivars in the UK are the cvs Cara and Pentland Javelin (Evans & Haydock, 2000). Whitehead (1998) reported also full resistance of the early potato cv. Rocket and of the maincrop cultivars Santé, Nadine and Valor to *G. rostochiensis* pathotype Ro1 and partial resistance of these four cultivars to *G. pallida* pathotype Pa2/3. Turner (1989) described *S. vernei* as the most commonly exploited source of *G. pallida* partial resistance. Resistance lines of *S. tuberosum andigena* have also been used to provide new cultivars with (partial) resistance to *G. pallida* (Whitehead, 1998). Whitehead (1991) reported that cv. Santé reduced populations of *G. pallida* by almost 88% except where virulent strains of *G. pallida* were present.

Research towards the development of cultivars with full resistance to *G. pallida* is ongoing and some positive results have been recently obtained. Genetically modified potato plants may be a solution as demonstrated by Urwin *et al.* (2000), who found that the transgenic *S. tuberosum* cv. Désirée expressing proteinase

inhibitors proved resistant to both *Globodera* species. Turner *et al.* (2000) reported that the potato clone 'Karaká' proved to be fully resistant to both PCN species and in addition it produced a harvestable crop of potatoes. A negative parameter in programmes of breeding for resistance to *G. pallida* is the variation in virulence exhibited by the different *G. pallida* populations, which results in often the resistance being broken down by the most virulent genes (Section A.1.5). Trudgill (2000) found that clones derived from *S. andigena* (e.g. CPC 2802) had greater and more consistent resistance to *G. pallida* than those derived from *S. vernei*, thus emphasising that breeding should concentrate on the former. He also observed that the repeated growing of these clones selected for increased virulence in *G. pallida* and he concluded that one population of *G. pallida* should be used to routinely screen for resistance. Earlier, at the molecular level, Van Der Voort *et al.* (1999) identified that the natural resistance gene *Gpa2* had a role in resistance against *G. pallida*. However, such natural resistance genes are effective over a very narrow host range, thus making their adoption into transgenic programmes extremely difficult (Urwin *et al.*, 2000); manipulation of these natural resistance genes in the future may prove helpful to overcome such problems (Atkinson *et al.*, 1998).

Evans & Haydock (2000) reported that Ro1-resistant cultivars represent approximately 43% of the total ware plantings in the UK, but only 6% of them have even partial resistance to *G. pallida* (Table A.7). The widespread use of cultivars with full resistance to *G. rostochiensis* has applied a selection pressure that has resulted in UK PCN populations evolving to be mainly *G. pallida* (Section A.1.2).

**Table A.7. Potato cultivars grown on over 2000 ha in the UK in 1999 and their resistance towards potato cyst nematodes (Source: British Potato Council; Evans & Haydock, 2000)**

Cultivar	% of total ware area * <sup>1</sup>	Resistance status		
		Ro1 * <sup>2</sup>	Pa2/3 * <sup>3</sup>	Susceptible
Maris Piper	21.2	✓		
Estima	9.9			✓
Cara	5.9	✓		
Saturna	5.0	✓		
Pentland Dell	4.3			✓
Nadine	4.3	✓	✓	
Hermes	4.1			✓
Désirée	4.1			✓
Marfona	3.5			✓
Lady Rosetta	3.3	✓		
Maris Peer	2.3			✓
Première	2.2	✓		
Maris Bard	2.2			✓
Wilja	2.0			✓
King Edward	1.9			✓
Russett Burbank	1.9			✓
Santé	1.4	✓	✓	
Total	79.5%	43.3%	5.7%	36.2%

\*<sup>1</sup> = total area of ware potatoes grown in the UK in 1999 was 146479 ha

\*<sup>2</sup> = fully resistant

\*<sup>3</sup> = partially resistant

#### A.1.10.4 Chemical control

Chemicals used to control PCN are divided into two broad categories: fumigant and non-fumigant (granular) nematicides. A soil fumigant can be applied at any stage

during crop rotation in the absence of a host crop when soil conditions are suitable, while the non-fumigants, granular nematicides are incorporated into the soil immediately before the crop is planted (Haydock & Evans, 1998).

Most of the non-fumigant (granular) nematicides are organophosphates and carbamates. These chemicals are powerful anticholinesterase inhibitors (Whitehead, 1998). Their principal mode of action is by nematostasis, i.e. they act by paralysing or disorientating the hatched J<sub>2</sub>s which are consequently unable to locate and invade the roots of the host plant (Hague & Pain, 1970). Because of their mode of action, these nematicides are often referred as 'nematostats'.

Phytotoxicity is rare among these compounds but they are highly toxic to man and other mammals (Franco, 1986). Because granular nematicides are non-phytotoxic they can be applied at planting without posing a risk to the crop. Granular nematicides have many advantages over fumigant nematicides: they are target-specific, they lack phytotoxicity and they are applied easily and at relatively low rates (Smelt *et al.*, 1996; Ambrose *et al.*, 2000).

Organophosphates are lipophilic and adsorbed by soil organic matter; to be effective against PCN they require intimate mixing with the soil (Whitehead, 1998). The soil type influences the effectiveness of an organophosphate nematicide. They are usually ineffective in controlling PCN populations in soils with high organic matter, but they have been proven to be highly effective in sandy and silty loam soils (Whitehead, 1998). At present, ethoprophos (Mocap 10G, Dow AgroSciences, UK) and fosthiazate (Nemathorin 10G, Ishihara Sangyo Kaisha Ltd, Japan) are the two organophosphate nematicides used commercially in the UK for PCN control (Whitehead 1998; Woods *et al.*, 1999).

The most widely used carbamate nematicides are aldicarb (Temik 10G, Bayer CropScience Ltd, UK) and oxamyl (Vydate 10G, DuPont de Nemours, USA). In the UK, the maximum commercial application rates are 33.6 kg ha<sup>-1</sup> for the former and 5.6 kg ha<sup>-1</sup> for the latter. These products are highly effective against *G. rostochiensis* but are less effective against *G. pallida* (Section A.1.7.3, Table A.2) and this is thought to be partly because *G. rostochiensis* reaches peak hatch levels earlier than *G. pallida* during the growing season, before the nematicides break down to ineffective concentrations in the soil (Whitehead, 1998). According to Evans (1993), hatch of *G. rostochiensis* is largely completed within a 6-week period, whereas in *G. pallida* it may take up to 12 weeks.

The rate of degradation of granular nematicides can vary dramatically in the field, as it depends on factors such as soil moisture, temperature, texture and pH (Leistra *et al.*, 1980; Whitehead, 1998). The activity of soil microorganisms might speed the breakdown of granular nematicides (Whitehead, 1998). Therefore, the rate of nematicide degradation would be expected to be slower in dry soils than in moist soils because of the reduced microbial population supported by the former; this has been demonstrated by Gerstl (1984) for the nematicide oxamyl. Gerstl (1984) also found that the rate of degradation of oxamyl was faster at 35°C compared with 15°C. Ambrose *et al.* (2000) studied the decay rate of oxamyl in 10 different commercial potato fields in Shropshire, UK, and found that the half-life of this nematicide ranged from 7 to 28 days. The half-lives of widely used nematicides is presented in Table A.8. The variations in the rates of degradation of the nematicides aldicarb and oxamyl in soils of different texture has been attributed to variations in the pH of the soils (Smelt *et al.*, 1979; Ambrose *et al.*, 2000). In general, the rate of breakdown is lower in organic soils with low pH, compared to loam soils with a high pH (Smelt &

**Table A.8. Half-lives ( $D_{t50}$ ) of nematicides in moist soils at 15°C  
(adapted from Smelt & Leistra, 1992)**

Active ingredient	Trade name	$D_{t50}$ (Days)	Reference
aldicarb	Temik	2-9	Bromilow <i>et al.</i> (1980)
oxamyl	Vydate	7-28	Ambrose <i>et al.</i> (2000)
carbofuran	Furadan	20-167	Ahmad <i>et al.</i> (1981)
ethoprophos	Mocap	16-120	Smelt <i>et al.</i> (1981)
fenamiphos	Nemacur	5-18	Lee <i>et al.</i> (1986)

Leistra, 1992). The different response of the two PCN species to granular nematicides application stresses the importance of correct identification of the PCN species (Section A.1.5) before any nematicide application (Evans & Haydock, 2000). Nematicide persistence is particularly important when the field is infested with *G. pallida* (Ambrose *et al.*, 2000).

Because granular nematicides cost the ware potato grower in the UK a minimum of £300 ha<sup>-1</sup> (up to £1000 for two applications) and degrade very rapidly in the soil, it is important that they are used effectively. Woods & Haydock (2000) found that best PCN control and yield protection were obtained when the nematicide granules were incorporated to a depth of *c.* 15 cm with tubers planted not deeper than the nematicide in the soil; shallow and deep incorporation depths (< 5 cm and > 35 cm, respectively) gave no benefits to the potato crop. These authors also suggested that, for any benefits to be gained in PCN population control and yield response, granular nematicides must be placed as uniformly as possible with the soil volume that can be exploited by the potato roots in the first 6 weeks after planting (target zone: 20-30 cm in diameter in the top of the ridge). Therefore the method of incorporation is also important; Smith & Bromilow (1977) showed that in peat fen

soils incorporating the nematicide granules by rotavators (L-bladed and spiked) resulted in higher yields and lessened PCN multiplication, as compared to when harrows were used.

Fumigants currently available for PCN control include two halogenated aliphatic hydrocarbons (methyl bromide, 1,3-dichloropropene) and two methyl isothiocyanate liberators (metham-sodium, dazomet) (Whitehead, 1998). Methyl bromide ( $\text{CH}_3\text{Br}$ ; trade names D-D and Vidden D) is a popular soil fumigant used in the control of PCN as well as of other soil pests and pathogens. However, this product, because of its gas formulation, poses health and environmental risks (Whitehead, 1998) and it was withdrawn from sale in the USA in 2001, a move possibly to be followed by other countries too (Ohr *et al.*, 1996; Grove & Haydock, 2000). Haydock & Evans (1998) reported that, in the UK, the fumigant nematicide 1,3-dichloropropene (Telone II), applied alone or with granular nematicides as part of an integrated management plan, can kill up to 80% of PCN eggs with just one application. This is achieved as gas percolates through the soil and kills the eggs while they are still in their protective cysts. Although fumigants can kill up to 80% of the PCN eggs, a greater kill may be needed to prevent an increase in the nematode population (Whitehead, 1986). Grove & Haydock (2000) conducted *in vitro* experiments to determine the level of 1,3-dichloropropene toxicity to *G. pallida* and *G. rostochiensis* and they found that it was highly toxic to both PCN species at dosages below the UK commercial application rates. The efficacy of fumigant nematicides in controlling PCN populations in the soil is affected by the method of application (Whitehead, 1998), the speed of the gaseous diffusion (rapid diffusion in high temperatures; Hague & Gowen, 1987) and the soil moisture (effectiveness decreases as soil moisture increases; Whitehead, 1998). For a fumigant nematicide to



be effective in controlling PCN populations, the soil surface should be smeared in salty or sandy soils or covered by polythene (Whitehead, 1998). Some fumigant nematicides are too expensive for use on potatoes (e.g. Dazomet and D-D) (Whitehead, 1998) and the majority of them are not only phytotoxic but also toxic to humans and to many non-target organisms, such as beneficial fungi and bacteria (Franco, 1986). Moreover, fumigant nematicides may cause groundwater contamination (Peoples *et al.*, 1980). As a result, the use of many fumigant nematicides was banned during the 1980's and non-fumigant nematicides dominate now the world nematicide market, with over 50% of the total market share (Nordmeyer, 1990). In the UK, the ratio of sales of granular to fumigant nematicides in 1999 was approximately 13:1 (Table A.9; Evans & Haydock, 2000).

Despite the extensive usage of nematicides, the increasing public concern about pesticide residues in food products and the strict limitations on nematicides use set by supermarkets (Haydock & Evans, 1998) have led nematologists to seek alternative methods to control PCN, possibly by combining different control strategies in IPM systems.

#### **A.1.10.5 Trap cropping**

Trap cropping is a method used to control cyst nematodes, where host plants are grown in the field for a period necessary to induce nematode hatch (and possibly root invasion; Whitehead, 1998) but not long enough to allow nematodes to multiply (Whitehead, 1977). The crop is destroyed and the haulm is removed from the field after nematodes have completed a few weeks of feeding time but before they start to multiply. According to Whitehead (1992) and Haydock & Evans (1998) the ideal trap crop is a crop that is established rapidly, is left to grow only for 6 or 7 weeks at

the most (ideally the crop should be removed as soon as the first PCN females are visible on the surface of the older roots), is tolerant to PCN attack and produces very large root systems, thus promoting maximum emergence of J<sub>2</sub>s from cysts in the soil. Such crops are then lifted and the plants are composted at the side of the field or, alternatively, on a waste ground to kill off the developing nematodes (Haydock & Evans, 1998).

Brodie (1982) reported that trap cropping as a method of PCN control is much more effective when resistant cultivars are used compared to susceptible ones. However, commercial potato cultivars are not so widely used as trap crops for PCN control because of the high costs involved (e.g. expense of tubers, cost of planting and destroying the crop in terms of labour; Whitehead, 1977) and the risk of increasing nematode populations when susceptible cultivars are grown or when the timing is wrong (i.e. after fertilisation occurs; Evans & Haydock, 2000).

**Table A.9. Areas treated annually with granular nematicides for PCN control in the UK (data up to 1999; Evans & Haydock, 2000)**

Active ingredient	Product	Market share (%)	Area treated (ha)	Value (£)
Aldicarb	Temik	49	13720	4074840
Oxamyl	Vydate	44	12320	3930080
Fosthiazate	Nemathorin	6	1680	536760
Ethoprophos	Mocap	1	280	131040

A successful use of trap cropping against *G. pallida* is described by Whitehead (1992), who found that the very tolerant cv. Cara induced hatch of up to 84% of the eggs within two months. The percentage reduction in PCN populations in very tolerant cultivars, such as Cara, is largest in heavy infestations and this could be

attributed to the stimulation of root production in heavy infestations by such cultivars, which would increase the production of PRL and hence, hatching (Whitehead, 1998). A trap crop and one application of the nematicide ethoprophos reduced *G. pallida* levels in the soil by 98.5% (Mugniery & Balandras, 1984). Haydock & Evans (1998) reported that, as with other PCN control tactics, trap cropping is more effective against *G. rostochiensis* than *G. pallida*.

A new approach involves the use of wild *Solanum* spp. as trap crops. Scholte (1999) investigated the possibility of controlling PCN by using non-tuber bearing Solanaceae as trap crops and found that *S. nigrum* and *S. sisymbirifolium*, used within crop rotation of highly resistant cultivars, significantly reduced the PCN population levels. More recently, *G. pallida* was successfully controlled using fully resistant (to both *Globodera* spp.) Solanaceae potato clones as trap crops (Table A.10; Turner *et al.* 2000).

#### **A.1.10.6 Integrated Pest Management**

In contrast with *G. rostochiensis*, the traditional PCN control strategies, which include use of nematicides (mainly granular; Section A.1.10.4), rotation (Section A.1.10.2), use of resistance cultivars (Section A.1.10.3) and trap cropping (Section A.1.10.5), have so far failed to limit or to prevent *G. pallida* population increases in the field and, as a result, UK potato growers face a serious *G. pallida* problem (Evans & Haydock, 2000). The current status in the control of *G. pallida* in the UK is such that fumigant nematicides (Section A.1.10.4), such as 1,3-dichloropropene (Telone II), are being re-introduced (Haydock & Evans, 1998), because they do not seem to work selectively on this species (Evans & Haydock, 2000). The realisation recently that the two PCN species have different biological characteristics (Section

A.1.7.3, Table A.2) and consequently, would respond differently to the various control tactics employed may help to achieve a better *G. pallida* control in the future. Nevertheless, since most of the conventional methods are ineffective against *G. pallida* when used separately, these and other methods must be combined into integrated control programmes if better results are to be obtained (Whitehead, 1998). Spaul et al. (1987) reported that the three main elements of PCN control (host plant resistance, rotation and nematicides) should be integrated because each acts at a different stage in the life cycle of *G. pallida* and hence has only a quantitative effect.

**Table A.10. (a) Reduction in *Globodera pallida* population size using resistance Solanaceae potato clones as trap crops and (b) Advantages of these clones over conventional trap crops (adapted from Turner et al., 2000)**

(a) <i>G. PALLIDA</i> POPULATION CHANGE		(b) ADVANTAGES OF (1), (2) & (3)
<u>Trap crop</u>		
(1) CPC 2488A	↓ 26%	✓ High levels of PCN hatch stimulation
(2) L3783/14	↓ 54%	✓ Highly tolerant
(3) Karaka	↓ 36%	✓ Application of nematicide may not be needed
(4) Cara (susceptible)	↑ 427%	✓ Highly resistant to all PCN pathotypes
(5) Fallow (no crop)	↓ 19%	

The classic integrated approach used by farmers in the UK includes application of a granular nematicide plus rotation (Hancock, 1988), which is often too short (1 in 3 and 1 in 4 are common; Haydock & Evans, 1998) to prevent the build up of PCN (*G. pallida* in particular) on ware land. On the other hand, longer breaks of up to 7 or 8 years between potato crops make crop rotation unprofitable for the grower. Haydock & Evans (1998) suggested that an alternative to this long wait is to reduce PCN populations more rapidly (by up to 80%) with one application of a soil

fumigant (e.g. Telone II) and a well-managed trap crop. Alphey *et al.* (1988) suggested that a reasonable interval between potato crops followed by the use of partially resistant cultivars and one application of nematicide offers the possibility of a more effective *G. pallida* control. Recent advances in the development of more effective *G. pallida* control practices, include the manufacture of computer programmes for modelling the integrated control of PCN (Elliott *et al.*, 2000).

In practice, the implementation of any integrated PCN control programme in the UK is influenced by the major supermarkets, which, in collaboration with the National Farmers' Union (NFU), have produced an integrated crop management protocol (ICM) for fresh market potatoes (Haydock & Evans, 1998). This protocol specifies an integrated approach to PCN management and involves pre-cropping sampling to determine species, viability and infestation levels, and the use of a resistant cultivar combined with an application of a nematicide (when necessary) and between cropping intervals of at least 5 years (Haydock & Evans, 1998).

#### **A.1.10.7 Biological control**

##### **A.1.10.7.1 The need for biological control**

The increasing concern about the effects of nematicides on human health and the environment combined with the limited effectiveness of these products against *G. pallida* has increased the amount of research into the development of more environmentally friendly methods of PCN control. One such method involves the deployment of biological control agents (BCA) which are antagonists of PCN. For a BCA to be successful against PCN, it should reduce the nematode population size to an average level that is lower than would occur in its absence (Stirling, 1991).

#### **A.1.10.7.2 Types of biological control**

Kerry (1987) classified biological control into natural and induced. In natural biocontrol, populations of BCA are increased without being specifically introduced, while in induced biocontrol, BCA are released in the field by man. There are two approaches to induced biocontrol: (i) inundation (microbial pesticide applications), where the BCA is introduced in large numbers to rapidly control a pest, but because it has a short persistence in soil frequent applications are required, and (ii) introduction (mass release) in soils, where the BCA is spread and established itself in soil to give a long-lasting control, without requiring a second application.

#### **A.1.10.7.3 Effectiveness of biocontrol against the potato cyst nematodes**

Although there have been several reports of successful PCN biocontrol *in vitro* or in pots, there is little evidence that these organisms are effective in the field (Whitehead, 1998). This is possibly because of the limited period in the PCN life cycle during which the nematodes are vulnerable to a BCA (Stirling, 1991); this period is more difficult to monitor under field conditions than in laboratory and glasshouse experiments. Potato cyst nematodes are well-adapted to living in the soil environment (Jones *et al.*, 1998; Devine, 2000) and so, in addition to other functions (Sections A.1.7.1, A.1.7.2 and A.1.7.4) they have developed defence mechanisms against possible BCA attacks (e.g. formation of the protective cyst surrounding the eggs, eggshell of unhatched J<sub>2</sub>s; Stirling, 1991). Kerry (1990) emphasised that biological control of PCN should not be seen as replacement for the use of nematicides because it does not provide a rapid kill of the pest. Haydock & Evans (1998) suggested that BCA, providing that they are applied at the time of planting

potatoes, would possibly be established on the crop's root system, thus preventing a large proportion of developing nematodes from reaching maturity.

The effectiveness of BCA in controlling PCN in the field may be increased when naturally suppressive soils are exploited and manipulated (Kerry, 1990). A naturally-suppressive soil occurs when a susceptible cultivar is grown intensively over a number of years without the implementation of control measures, thus allowing the populations of PCN antagonists to build-up (Crump, 1989). However, this strategy is rarely followed, because it is economically unprofitable for the potato grower in the short term (Kerry, 1990) and because PCN populations are usually not allowed to reach the levels required for natural decline to occur (Crump, 1989). Due to these limitations, amendments (organic and inorganic) are sometimes applied to the soil in order to enhance the activity of indigenous microbial populations (Stirling, 1988).

#### **A.1.10.7.4 Microbial antagonists of potato cyst nematodes**

Potential soil-borne PCN antagonists include parasitic (endoparasitic fungi of females and eggs; Kerry, 1987) and non-parasitic microorganisms (rhizosphere bacteria and mycorrhizal fungi; Sikora, 1992).

Most of the research on biological control of PCN has focused on endoparasitic fungi of females and eggs (Jatala, 1986). The advantage of these organisms over other nematophagous fungi, which parasitise J<sub>2</sub>s, is that they target the egg-producing stage of the nematode life cycle (Crump, 1989) and hence, they may be applied over a longer period of time (e.g. even in spring and winter when the eggs are dormant). Female parasites as BCA of PCN have also attracted the interest of nematologists because female nematodes play a very important role in PCN population dynamics.

Crump (1989) reported a 57% control of *G. pallida* in pots with artificially cultured *Verticillium chlamydosporium*. In another pot trial, *Colletotrichum coccodes* reduced the egg population of *G. rostochiensis* by 79% and of *G. pallida* by 58% (Crump, 1989). Under field conditions, satisfactory results, in terms of PCN control using fungal parasites, have been obtained with *Paecilomyces lilacinus* (41-54% reduction in the number of eggs and females of *G. rostochiensis*; Stirling, 1988) and with *Acremonium sordidulum* (reduction of the population of newly formed *G. pallida* cysts by nearly 64%; Sikora, 1992).

The other types of nematophagous fungi, which include endoparasitic fungi of vermiform nematodes and nematode-trapping fungi (Kerry, 1987), have proven to be ineffective against the cyst nematodes (Gray, 1988). *Pasteuria penetrans* is an obligate bacterial parasite of the root-knot nematodes *Meloidogyne* spp. with considerable potential as a BCA (Kerry, 1987; Chen & Dickson, 1998); Birchfield & Antonopoulos (1976) reported that it reduced *Meloidogyne* populations in pots by 99% within only 3 weeks. However, this BCA has not been exploited commercially because of difficulties in producing the high levels of inoculum required to offer protection in the field. Another *Pasteuria* isolate, *P. nishizawae*, a mycelial and endospore-forming bacterium, is parasitic on the cyst nematodes *Heterodera* and *Globodera* spp. (Sayre *et al.*, 1991; Chen & Dickson, 1998). In a microplot study conducted by Atibalentja & Noel (1999), *P. nishizawae* gave promising results as a BCA of the soybean cyst nematode (SCN) *H. glycines*. The authors concluded that given sufficient time following introduction into a field, *P. nishizawae* might build up to levels that would be effective as a component in an integrated SCN control strategy. Similar studies should be conducted in the future to test the effectiveness of this BCA against PCN as well.



Bacteria that colonise the rhizosphere are known as rhizobacteria (or rhizosphere bacteria). Some rhizobacteria have the ability to promote plant growth and are referred to as plant-growth promoting rhizobacteria (PGPR), while others improve plant health and hence are described as plant-health promoting rhizobacteria (PHPR) (Sikora, 1992). Apart from promoting plant growth and/or plant health, many rhizobacteria have shown potential to suppress plant root pathogens, including parasitic nematodes. Mechanisms by which rhizobacteria can control plant parasitic nematodes include egg hatch inhibition (Westcott & Kluepfel, 1993; Andreoglou *et al.* 2000), production of metabolic compounds toxic to nematodes (Meadows *et al.*, 1989), and degradation of HF (Oostendorp & Sikora, 1989). Of these mechanisms, egg hatch inhibition has attracted the interest of nematologists in relation to PCN biocontrol. Racke & Sikora (1992) demonstrated that isolates of the PHPR *Agrobacterium radiobacter* and *Bacillus sphaericus* reduced the hatching of *G. pallida* in glasshouse and field trials. Andreoglou *et al.* (2000) noted that the *in vitro* hatching of *G. rostochiensis* was inhibited and root invasion was prevented, after only 1 week of exposure to the symbiotic bacterium *Pseudomonas oryzihabitans*, from the entomopathogenic nematode *Steinernema abbasi*. Some rhizobacterial isolates have the ability to stimulate the hatch of one or of both PCN species *in vitro* by altering the production of hatching chemicals present in PRL (Section A.1.10.8). The other major category of root colonising microorganisms, which is arbuscular mycorrhizal fungi (AMF), can also antagonise the activity of plant parasitic nematodes (Section A.2.4) and affect PCN hatch (Section A.2.5).

#### A.1.10.8 Control via hatch stimulation

Because PCN hatch freely when stimulated by HF<sub>s</sub> produced in host root leachates (Section A.1.7), it has been considered that application of host leachates (or HF<sub>s</sub>) in the field could stimulate PCN hatch in the absence of the host crop (Perry, 1989; Nordmeyer, 1990; Devine, 2000). In such case, the hatched J<sub>2</sub>s would starve due to the lack of a suitable host, the PCN decline rate would be accelerated, and the next crop would benefit because of the reduction in the PCN population size. This may improve the effectiveness of the conventional methods employed in the control of *G. pallida*, which often fail to control this species due to its delayed hatch and slow decline rate (relative to *G. rostochiensis*; Section A.1.7.3). Hatching factors in PRL can be defined as semiochemicals, which are chemicals produced by one organism (in this case the potato plant) and affect the behaviour of another organism (i.e. PCN in this case); more precisely, the HF<sub>s</sub> are kairomones because it is the receiving rather than the producing organism that benefits (Devine, 2000). The hatch of cyst nematodes other than PCN has also attracted the interest of researchers. Masamune *et al.* (1982) isolated a HF called glycinoclepin A from kidney bean roots extracts, which stimulated the hatch of *H. glycines* at concentrations as low as  $10^{-12}$  g ml<sup>-1</sup> and subsequently, the HF<sub>s</sub> glycinoclepin B and C were purified by Fukuzawa *et al.* (1985).

Tsutsumi (1976) found that PRL, capable of inducing PCN hatch, persisted in soil for up to 100 days after the potato plants were removed. The persistence of PRL in soil was also suggested by Whitehead (1973) as a reason for the increased hatch of *G. rostochiensis* in the absence of the host crop. Perry & Beane (1982) reported that just five minutes exposure of *G. rostochiensis* to PRL were enough to trigger the hatching of this nematode. These reports led Perry (1989) and Evans (1993) to

speculate that it may be feasible to control PCN by applying host root leachates and/or HFs (natural or artificial) in the field as a novel PCN control method. Whitehead (1992) found that the artificial hatching agent sodium metavanadate ( $\text{NaVO}_3$ ) at a concentration of  $10^{-3}$  M stimulated the emergence of *G. pallida* in bare fallow soil but did not improve the control of *G. pallida* by oxamyl when it was applied to potato seedbeds. Devine (1994) found that *in vivo* application of raw unconcentrated PRL to artificially infested soil gave a 72% hatch of *G. rostochiensis* eggs and this was increased further to 82% with the application of partially-purified PRL. Devine & Jones (2000) reported that the exogenous application of HFs in the field reduced the population size of *G. rostochiensis* by approximately 50%, which was markedly higher than that reported by Whitehead (1977), who used the artificial HF picrolonic acid (33% reduction). Devine *et al.* (2001) found that *G. pallida*-selective and -specific HFs were more mobile in soil with high organic matter than those of *G. rostochiensis*, suggesting that there may be a greater potential for controlling *G. pallida* than *G. rostochiensis* in fields with high organic matter using the 'suicide' hatch strategy.

Jones *et al.* (1998) suggested that the seasonal flushes of PCN hatch in fallow soil reported by Tsutsumi (1976) could have been the result of the production of HFs by soil microorganisms. There is increasing evidence for a role for microbial HFs in PCN hatch. Devine *et al.* (1996) reported that certain HFs normally present in PRL from conventionally grown plants were absent from aseptically produced PRL suggesting a role for microbes, perhaps rhizobacteria, in the production of HFs and therefore in hatch stimulation of PCN. Carroll (1995) found that certain bacteria isolated from the potato rhizosphere increased PCN hatch (the effect was PCN species-specific) when grown in PRL, whereas in the absence of PRL, these isolates

had no effects on PCN hatch. According to Ryan (2003) this might have been the result of the production of HFs by the bacterial isolates. Cronin *et al.* (1997) demonstrated that 2,4-diacetylphloroglucinol (DAPG), produced by *Pseudomonas fluorescens*, increased the hatch of *G. rostochiensis* *in vitro* and in pot experiments. More recently, Ryan (2003) demonstrated that PRL from non-sterile grown microplants contained more HFs, and produced more HSs, but fewer HIs, than the PRL from sterile grown microplants.

## **A.2 Mycorrhizal Fungi**

### **A.2.1 Introduction**

Mycorrhizae are highly evolved, symbiotic associations developed between plant roots and soil fungi. Mycorrhizal fungi are ubiquitous in soils throughout the world and many important plants of agriculture, forestry and horticulture are dependent on mycorrhizae (Linderman, 1988). The partners of the mycorrhizal association include most vascular plants and members of the fungal kingdom (Basidiomycetes, Ascomycetes and Zygomycetes; Brundrett, 1991). Brundrett *et al.* (1994) described this association as a three-way interaction involving host plants, mutualistic fungi and soil factors.

### **A.2.2 Types of mycorrhizae**

Several different types of mycorrhizal associations have been recognised but the majority of them fall into two major categories. These are the ectomycorrhizae and the endomycorrhizae.

Ectomycorrhizae (from ‘ecto’, (GK): outside) are associations formed between fungal species of the Basidiomycetes and Ascomycetes and plant species of the

families Pinaceae, Betulaceae, Fagaceae, Salicaceae and Myrtaceae (Linderman, 1988). In the ectomycorrhizae, the fungus grows inside or between plant root cells and forms a hyphal network (called 'hartig net') covering the plant root (Quarles, 1999). These associations are formed predominantly on the fine root tips of the host (Brundrett *et al.*, 1994).

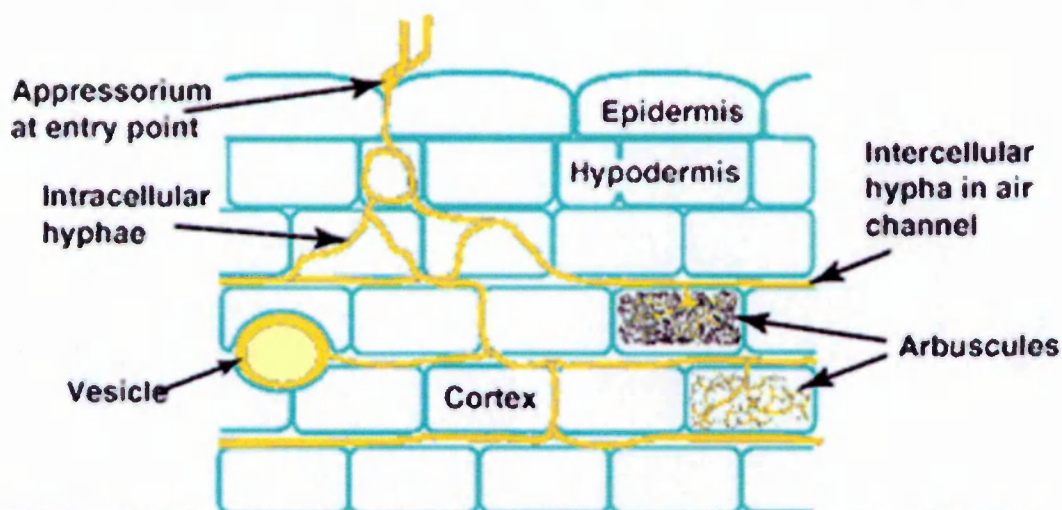
Three types of endomycorrhizae (from 'endo', (GK): inside) have been recognised: (i) orchid mycorrhizas, (ii) ericoid mycorrhizas, and (iii) vesicular-arbuscular mycorrhizas (Linderman, 1988). Of these three types, vesicular-arbuscular mycorrhizas will be described here in detail.

Vesicular-arbuscular endomycorrhizal fungi (abbreviated as AM, VAM or AMF), so called because of the production of vesicles and arbuscules within root cortical cells (Linderman, 1988), are associations formed between a wide diversity of plants and fungi in the Zygomycetes order Glomales, family Endogonaceae (Brundrett *et al.*, 1994). Genera of AMF fungi include *Glomus*, *Gigaspora*, *Acaulospora*, *Sclerocystis*, *Scutellospora* and *Entrophospora* (Table A.11; Morton & Benny, 1990). The association starts when hyphae from root fragments in soil respond to the presence of a root by growing towards it. The molecular basis of the establishment of AMF symbiosis is largely not understood. It is speculated that root infection by AMF is initiated when the root releases signal, flavonoid-like, molecules, which are recognised by the fungal spores and hyphae and they start germinating (spores) and growing (hyphae) towards the root surface (Linderman, 1988; Sahay *et al.*, 1998). In potato, Rausch *et al.* (2001) identified the phosphate transporter gene StPT3 in root sectors where mycorrhizal structures are formed, suggesting that the mutualistic symbiosis evolved by genetic rearrangements in the StPT3 promoter. The association may also be initiated by spore germination;

however, hyphae resulting from spore germination have limited capacity to grow and if they do not find a susceptible root within a week they will die (Brundrett *et al.*, 1994). The fungus then establishes contact with the root and grows along its surface. Following this, one or more hyphae produce swellings called appressoria (Fig. A.9), with the aid of which they penetrate epidermal root cells. The hyphae cross then the hypodermis through passage cells and start branching in the outer cortex, which they subsequently penetrate (Brundrett *et al.*, 1994) (Fig. A.9). Once inside the cortex, hyphae proliferate and develop intra- or inter-cellularly (Quarles, 1999). Growth of hyphae within the inner cortex results in the formation of arbuscules (Fig. A.9), which are highly branched haustoria formed on hyphae branches in cortical cells (Quarles, 1999). Arbuscules start to form approximately 2 days after hyphae penetrate the root and are the major site of exchange between the fungus and the plant (Brundrett *et al.*, 1996). Once arbuscules have been formed, they penetrate the host root cell walls and invaginate the plasmalemma (Brundrett *et al.*, 1994). Soon after the first arbuscules are formed, AMF form vesicles in the root cortex (Fig. A.9). Vesicles are globose bodies caused by a terminal swelling of a hypha of the mycorrhizal fungus and contain lipids and cytoplasm (Brundrett *et al.*, 1994). They develop to accumulate storage products in many AMF associations (Hussey & Roncadori, 1982). Vesicles are located in the inner or outer layer of the cortical parenchyma (Gianinazzi-Pearson *et al.*, 1995). Some of the growing hyphae form an extensive network that projects into the surrounding soil (Quarles, 1999).

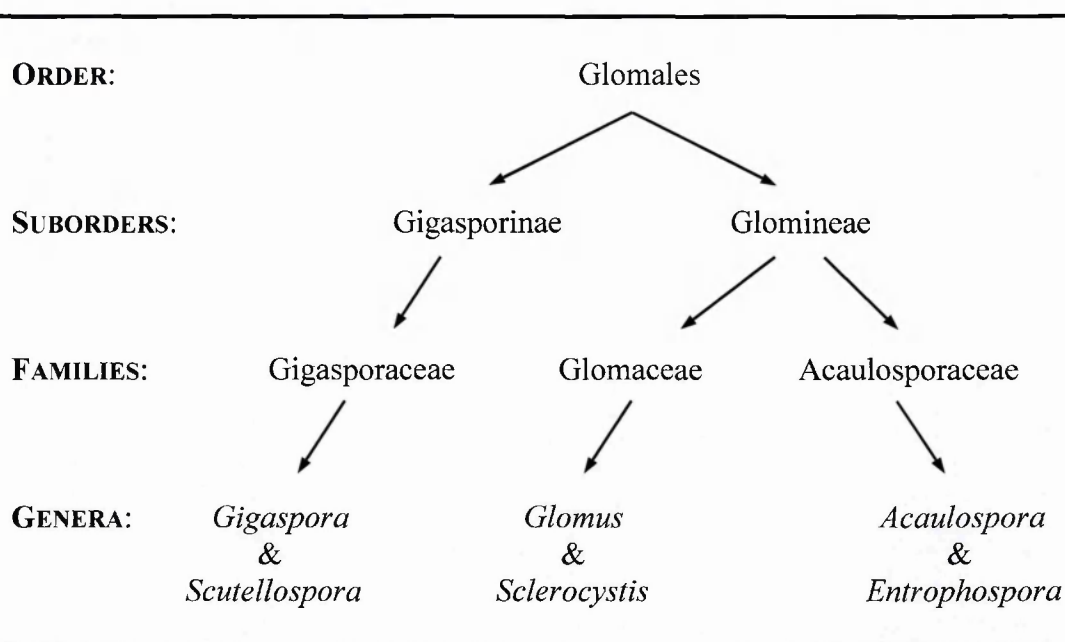
### **A.2.3 Benefits from arbuscular mycorrhizas**

Both the plant and the fungus benefit from the arbuscular mycorrhizal association; the fungus gathers essential nutrient elements from the soil and transfers



**Fig. A.9.** Developmental stages in root colonisation by arbuscular mycorrhizal fungi  
(Credits: M. Brundrett, Australian Centre for International Agricultural Research)

**Table A.11. Classification scheme of Morton & Benny (1990) for arbuscular mycorrhizal fungi**



them to the plant, and in exchange, the plant feeds the fungus with sugar and other nutrients obtained through photosynthesis (Quarles, 1999). Although mycorrhizal fungi are ubiquitous in soils throughout the world and the large majority of plant species belong to genera that form AMF and other types of mycorrhizal fungi associations, the degree of dependence on mycorrhizal associations varies greatly

among plants (Janos, 1980; Brundrett, 1991). Janos (1980) defined the term “mycorrhizal dependency” as follows: “A measure of the benefit provided by mycorrhizas and will depend on relative contribution of root and mycorrhizal mediated nutrient uptake to plants”. Plant species generally either have (a) consistently high levels of mycorrhizas (obligatorily mycorrhizal), (b) intermediate, or variable levels of mycorrhizas (facultatively mycorrhizal), or (c) are not mycorrhizal (Janos 1980, Brundrett, 1991). A common trend in mycorrhizal evolution is from obligatorily AMF to facultatively AMF and then to non-mycorrhizal, while the reverse situation where non-mycorrhizal plants re-acquire AMF is very unusual (Brundrett, 2002). Facultative mycorrhizal plants can be grown without AMF if sufficient nutrients are available and the benefits to plants are conditional on soil fertility (Brundrett, 2002).

In natural ecosystems, facultatively mycorrhizal or non-mycorrhizal plants are most abundant in harsh or disturbed habitats such as waterlogged, saline or arid soils where plant productivity is limited and mycorrhizal fungi would be of limited benefit (Brundrett, 1991). Under such conditions the exclusion of mycorrhizal fungi would conserve energy to the plant (Brundrett, 2002). The principle characteristic of non-mycorrhizal roots is the capacity to exclude AMF (Brundrett, 2002). The mechanism by which non-mycorrhizal roots exclude glomalean fungi is not totally understood. Giovannetti & Sbrana (1998) suggested that non-host roots possibly fail to trigger those fungal genes responsible for attracting AMF, while according to Brundrett (2002) it is more likely that AMF attempt colonisation but are blocked by defence reactions of non-host roots. Non-mycorrhizal plants have evolved separate techniques than mycorrhizal plants in order to obtain their nutrients and survive. In particular, they have evolved specialised root systems (e.g. cluster roots, different



patterns of root hair production), which secrete organic compounds to modify the soil pH so as to increase nutrient availability (Skene, 1998; Pemberton *et al.*, 2001; Brundrett, 2002). Apart from the 'true' non-mycorrhizal plants, i.e. plants that are non-hosts of AMF (families Amaranthaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Commelinaceae, Cyperaceae, Juncaceae and Polygonaceae; Brundrett, 1991), non-mycorrhizal mutants of AMF host plants also exist, e.g. in tomato (Gao *et al.*, 2001).

In agricultural ecosystems the traditional management practices, such as tillage, crop rotation, topsoil removal, fallowing and application of pesticides, fertilisers and lime may adversely affect AMF populations in the field (Sylvia, 1998). Pot and field trials with mycorrhizal-dependent plants has shown that in situations where native AMF inoculum potential is low or ineffective, as well as in infertile soils, providing the appropriate AMF can be beneficial for the plant. Under these conditions, AMF-inoculated plants may have numerous advantages over uninoculated plants. These can be summarised to the following:

- Increased plant nutrient supply and hence, enhanced plant growth, by extending the volume of soil accessible to the plant and by acquiring nutrient forms that would not normally be available to the plant (Brundrett *et al.*, 1994); in particular, AMF have shown to increase the supply of: (i) immobile elements such as P, Zn, and Cu, and (ii) mobile ions such as S, Ca, K, Fe, Mg, Mn, Cl, Br and N (Tinker, 1984). Since AMF feed the plant with minerals, AMF plants need less applied fertiliser (Quarles, 1999).
- Increased water uptake (Linderman, 1988), plant photosynthesis (Quarles, 1999) and hence, carbon assimilation (Brundrett *et al.*, 1994).

- Reduced drought stress by altering the physiology of the plant (Linderman, 1988).
- Reduced stress to high salt levels and heavy metals (Tinker, 1984).
- Increased resistance to soil-borne fungal pathogens due to changes in plant morphology and/or physiology (Dehne, 1982; Mark & Cassells, 1996).
- Reduced infestation or damage caused by plant parasitic nematodes due to stimulatory effects on root growth (Hussey & Roncadori, 1982).
- Increased yields (Ellis, 1998).
- Increased phytohormone production (i.e. cytokinins, gibberellins and ethylene; Linderman, 1988).
- Improved transplant survival and rate of plant establishment after transplanting (Barrows & Roncadori, 1977).

#### **A.2.4 Working with arbuscular mycorrhizas**

##### **A.2.4.1 Isolation and propagation**

Arbuscular mycorrhizal fungi are propagated by growing them with a host plant, such as *Trifolium* (with *Rhizobium* inoculum) or *Sorghum vulgare*, in soil pot cultures (Brundrett *et al.*, 1996). The substrate can be a coarse-textured soil (e.g. sandy soil) with moderate nutrient levels or calcined clay (B. Blal, BioRize, France, personal communication). In some cases, Glomalean fungi are propagated in aeroponic or other hydroponic systems (Jarstfer & Sylvia, 1993). Spores of AMF are separated from soil samples by wet-sieving and centrifugation (Brundrett *et al.*, 1996).

#### A.2.4.2 Inoculation methods

Mycorrhizal inoculum can be defined as a material that carries mycorrhizal fungi in a usable form to the intended host plants. In the case of AMF, the active ingredients consist typically of spores, hyphae, and colonised root fragments. Mycorrhizal inoculation is generally conducted after the roots are formed (exception: inoculation of non-rooted microcuttings; Vestberg & Estaún, 1994). The AMF inoculum must be placed in the root zone, not on the soil surface, because in the latter case it will die quickly (especially if the soil temperature is high) and therefore it will be inaccessible to the growing roots (B. Blal, BioRize, personal communication). In micropropagated systems mycorrhizal inoculation can be conducted at any of the following three stages (Vestberg & Estaún, 1994):

- *In vitro*, at the rooting phase.
- *In vivo*, immediately after the rooting phase at the start of the acclimatisation period.
- *In vivo*, after the acclimatisation before the beginning of the hardening phase under glasshouse conditions.

In the cases above, the AMF inoculum is usually placed in the planting hole directly under the seedling, ensuring that physical contact between the AMF inoculum and roots is established (Ryan *et al.*, 2000).

In agricultural use, the mycorrhizal inoculum may be injected with fertiliser banding equipment, incorporated during the bed-forming process (T&J Enterprises, Bio-Vam Mycorrhiza), or applied by hand in the planting hole (e.g. in potato fields). Commercial AMF inocula intended for field applications normally come in a uniform carrier so that they will be compatible to mechanical handling (B. Blal,

BioRize, personal communication). In a field experiment, Koch *et al.* (1997) inoculated garlic by spreading the AMF inoculum to the planting furrow. In a pot experiment involving potato plants, McArthur & Knowles (1993) mixed the AMF inoculum with the soil (100 g AMF inoculum per 25-cm diameter pot) before planting the tubers. Aqueous AMF inocula are used as root dips for container seedlings and are too expensive for direct field application (T&J Enterprises, Bio-Vam Mycorrhiza).

#### A.2.4.3 Clearing and staining mycorrhizal roots

Mycorrhizal structures are often not visible in fresh root samples because they are obscured by the natural pigments and cell contents within roots (Brundrett *et al.*, 1994). Observation of internal mycorrhizal structures is only possible after roots have been cleared and stained, using chemical agents and a dye, respectively (Brundrett *et al.*, 1996).

Mycorrhizal root samples (preferably no more than 2 g) are usually cleared in a 10% (w/v) KOH solution, in an autoclave cycle of 15-20 minutes at 121°C (Phillips & Hayman, 1970). Alternatively, the roots can be cleared in a water bath by heating the KOH solution to 60-90°C for a minimum of 2 hours (roots from older plants or field-collected roots normally require longer clearing times than those from younger plants; Brundrett *et al.*, 1994).

Cleared roots are then captured on fine sieves and rinsed thoroughly with water before transferring into the staining solution. Roots can be stained with 0.03% (w/v) Chlorazol black E (CBE) (Brundrett *et al.*, 1994) or with 0.05% (w/v) Trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) (Kormanik & McGraw, 1982). The staining solution containing the mycorrhizal root samples is heated using the

same methodology as for clearing the roots. Alternatively, roots can be stained by leaving them in the solution for one or more days at room temperature (Brundrett *et al.*, 1994). These dyes bind to fungal hyphae, making them visible in high magnifications under a compound microscope. Staining quality is improved by destaining the roots in 50% (v/v) glycerol-water solution for several days prior to observation; this allows excess stain to leach from the roots (Brundrett *et al.*, 1996).

#### **A.2.4.4 Estimation of root colonisation by arbuscular mycorrhizal fungi**

The most frequently used method for measuring the degree of root colonisation by AMF is the gridline intersect method (Giovannetti & Mosse, 1980; Fig. A.10). Usually, a minimum of 100 intersections are required to assess one sample. Intersections with mycorrhizal structures (hyphae, arbuscules and/or vesicles) are recorded as being 'infected' and those without mycorrhizae as 'uninfected'. Observations are conducted under a dissecting microscope (x40 magnification). The Nicolson formula is used to estimate the percentage AMF root colonisation (Nicolson, 1960):

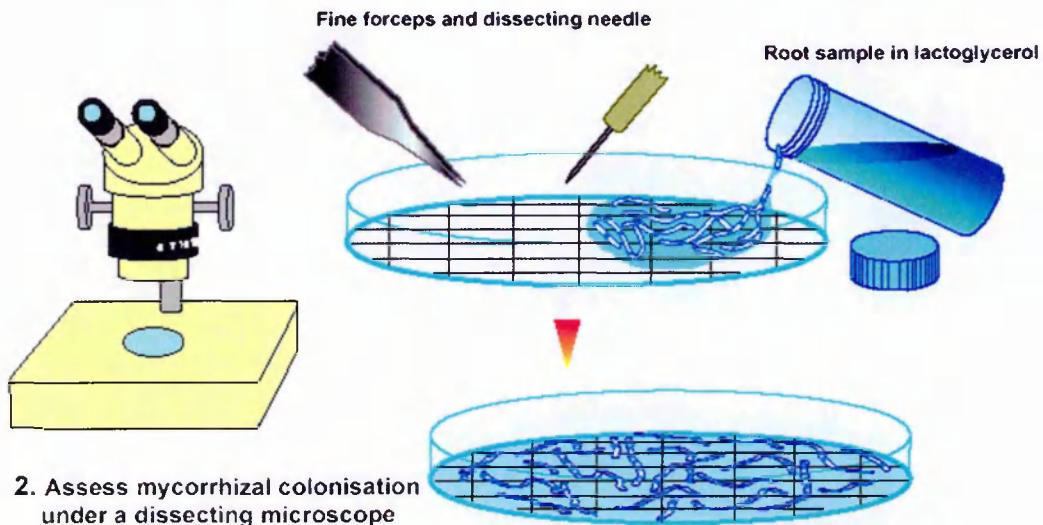
$$\% \text{ root colonisation} = \frac{\text{no. of infected intersections}}{\text{total no. of intersections}} \times 100$$

#### **A.2.4.5 Factors affecting the result of mycorrhizal inoculation**

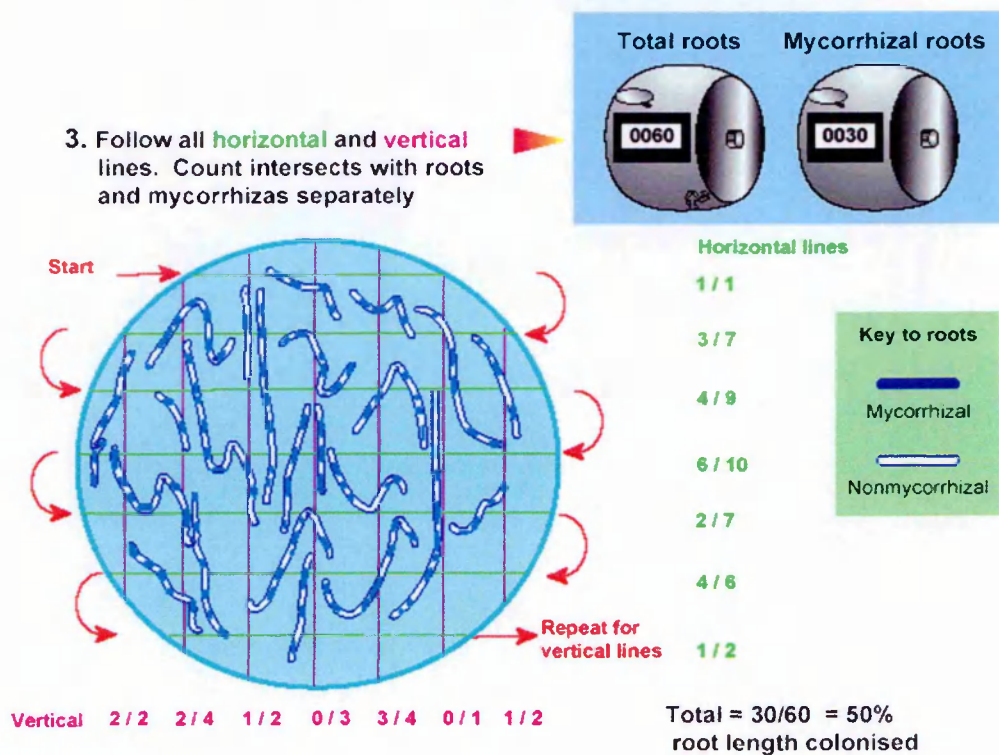
When inoculating plants with AMF it is very important to establish the objective of the inoculation, as this will influence not only the result of the mycorrhizal inoculation but also the selection of the inoculation procedure. The aim of AMF inoculation may be enhancement of plant growth, lower fertilisation input,

## THE GRIDLINE INTERSECTION METHOD

1. Randomly disperse cleared and stained roots in dish with grid lines



3. Follow all **horizontal** and **vertical** lines. Count intersects with roots and mycorrhizas separately



**Fig. A.10.** The gridline intersection method of Giovannetti & Mosse (1980) for counting the percentage root colonisation by arbuscular mycorrhizal fungi (Credits: M. Brundrett, Australian Centre for International Agricultural Research)

increased survival, and/or increased resistance to biotic and abiotic factors (Vestberg & Estaún, 1994). The result of mycorrhizal inoculation is affected by a wide range of factors, which can be one or more of the following (Linderman, 1988):

- Time and method of inoculation (Section A.2.3.2).
- Host-fungus specificity.
- Soil factors such as fertility, moisture levels, and physical properties.
- Environmental conditions such as light and temperature.
- Cultural practices such as foliage applications of chemicals.
- Soil-microbial interactions.

Although, in general, AMF have a wide host range, they can show specificity (Anderson, 1988). Quarles (1999) attributed this specificity to the fact that AMF spores tend to produce local strains, which help them to adapt to a specific climate, soil and host plant. Therefore, by selecting the most efficient fungal partner, a more successful symbiosis can be established, with subsequent benefits for the plant (Vestberg & Estaún, 1994).

Soil disturbance, such as agricultural tillage and erosion can also reduce the levels of AMF propagules (Habte *et al.*, 1988). Furthermore, excessive NaCl levels in soil inhibit mycorrhizal formation and restrict the activity of most AMF (Brundrett *et al.*, 1996).

Arbuscular mycorrhizal fungi have the potential to make successful cultivation possible at low rather than at high soil P levels by exploiting the released P more effectively (Brundrett *et al.*, 1996). Increase in soil P levels usually reduces the benefit provided by AMF to plants (Jones *et al.*, 1990). Therefore, the supply of phosphates, as well as of other nutrients, in soil plays a key role on the degree of

success of the plant-AMF symbiosis (Abbott & Robson, 1991). Phosphorus is the most important plant-growth limiting factor which can be supplied by AMF, because of the many abiotic and biotic factors which can restrict its mobility in soils (Hayman, 1983). One of the reasons for the reduction in the benefit provided by AMF to plants is the increase in soil P levels (Bougher *et al.*, 1990; Jones *et al.*, 1990; Schweiger *et al.*, 1995).

#### **A.2.5 Interactions between arbuscular mycorrhizal fungi and nematodes**

Endoparasitic nematodes, such as PCN, and AMF are commonly found inhabiting the rhizosphere and colonising the roots of the same plant (Siddiqui & Mahmood, 1995). Nevertheless, these two groups of microorganisms have opposite effects on plant growth (Hussey & Roncadori, 1982). Several studies have provided evidence that certain AMF act as biocontrol agents of plant parasitic nematodes (e.g. Dehne, 1982; Hussey & Roncadori, 1982; Smith, 1987). However, in some cases AMF colonisation had no effect on nematode development and in a few cases nematode levels increased following AMF colonisation (Hussey & Roncadori, 1982). The different types of AMF-nematode interactions and some selected examples are presented in Table A.12.

The reduction in nematode reproduction following inoculation of plants with AMF does not appear to be the result of nematode parasitism by the mycorrhizal fungi; only stressed or weakened eggs may be colonised by AMF (Siddiqui & Mahmood, 1995). A more likely scenario is that the effect of the nematode is reduced due to the increased plant growth in the presence of AMF (Pinochet *et al.*, 1996). Mycorrhizal fungi may increase the resistance and/or tolerance of the plant to nematode attack by inducing changes in plant root morphology and by causing



physiological, histopathological or biochemical changes to the plant (Siddiqui & Mahmood, 1995).

#### **A.2.6 Effects of arbuscular mycorrhizal fungi on potato cyst nematodes hatch**

Results of recent experiments have provided evidence that, in addition to rhizobacteria, AMF have also the ability to affect PCN hatch. Ryan *et al.* (2000) found that inoculation of the maincrop potato cultivar Golden Wonder with Vaminoc, a commercial mixed-isolate population of three *Glomus* spp., increased the early hatch of *G. pallida* *in vitro* and in-soil. It was later demonstrated that the increase in *G. pallida* hatch following mycorrhizal inoculation of potato roots was associated with increased production of HFs and HSs in the PRL from mycorrhizal plants, relative to non-mycorrhizal plants.

**Table A.12. Possible effects of interactions between plant parasitic nematodes and arbuscular mycorrhizal fungi (AMF) (Hussey & Roncadori, 1982) and examples of AMF effects on nematode reproduction (adapted from Siddiqui & Mahmood, 1995)**

TYPE OF INTERACTION	COMPONENT	EFFECT ON COMPONENT	
1. Neutral	Fungus	Root infection or sporulation not altered	
	Host	Mycorrhizal stimulation of vegetative growth or yield not altered; nematode suppression of vegetative growth or yield not offset	
	Nematode	Attraction to roots, penetration, or subsequent development and reproduction not altered	
2. Positive	Fungus	Root infection or sporulation increased	
	Host	Nematode suppression of vegetative growth or yield offset	
	Nematode	Attraction to roots, penetration, or subsequent development and reproduction suppressed	
3. Negative	Fungus	Root infection or sporulation suppressed	
	Host	Vegetative growth or yield response to mycorrhizae suppressed	
	Nematode	Attraction to roots, penetration, or subsequent development and reproduction increased	
HOST PLANT	NEMATODE	AMF	EFFECT OF INTERACTION ON NEMATODES
Potato	<i>Globodera rostochiensis</i>	<i>Glomus fasciculatum</i>	Adverse
Tomato	<i>Meloidogyne incognita</i>	<i>Glomus mosseae</i>	Adverse
Cotton	<i>Pratylenchus brachyurus</i>	<i>Gigaspora margarita</i>	Neutral
Onion	<i>Meloidogyne hapla</i>	<i>Glomus fasciculatum</i>	Neutral
Tobacco	<i>Heterodera solanacearum</i>	<i>Endogone gigantea</i>	Stimulatory
Cotton	<i>Meloidogyne incognita</i>	<i>Gigaspora margarita</i>	Stimulatory

### A.3 Research aims

- To identify AMF isolates that effectively colonise potato roots and have the ability to affect the hatch of the two potato cyst nematode species (*Globodera pallida* in particular)
- To investigate whether there is variation in the hatching activity of potato root leachates between mycorrhizal and non-mycorrhizal plants
- To investigate if plants treated with single AMF isolates have better effects than plants treated with mixed AMF preparations, in terms of PCN hatch and potato plant growth
- To determine the effects of applying both AMF and the nematicide aldicarb at planting on the hatch of *G. pallida*, multiplication rate of *G. pallida*, and yield of potatoes

The hypothesis was that:

- Application of selected AMF isolates on PCN-infested land planted to potatoes would increase the effectiveness of granular nematicides by accelerating the early hatch of *G. pallida*.

## A.4 References

- ABBOTT, L.K. & ROBSON, A.D. (1991). Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agriculture, Ecosystems and Environment* 35, 121-150.
- AGRIOS, G. N. (1997). Plant Pathology. Academic Press, San Diego, California, USA, 635 pp.
- AHMAD, N., WALGENBACH, D.D. & SUTTER, G.R. (1981). Degradation rates of technical carbofuran and a granular formulation in four soils with little known insecticide history. *Bulletin of Environmental Contamination and Technology* 23, 572-574.
- ALPHEY, T.J.W., ROBERTSON, W.M. & LYON, G.D. (1988). Rishitin a natural plant product with nematocidal activity. *Revue de Nématologie* 11, 399-404.
- AMBROSE, E., HAYDOCK, P.P.J. & WILCOX, A. (2000). Degradation of the nematicide oxamyl in field conditions. *Aspects of Applied Biology* 59, 41-51.
- ANDERSON, A.J. (1988). Mycorrhizae-host specificity and recognition. *Phytopathology* 78, 375-378.
- ANDREOGLU, F.I., GOWEN, S.R. & SAMALIEV, H.Y. (2000). Temperature response of the bacterium *Pseudomonas oryzihabitans*, from the entomopathogenic nematode *Steinernema abbasi*, on the potato cyst nematode *Globodera rostochiensis*. *Aspects of Applied Biology* 59, 67-73.
- ANON. (1969). Council Directive of 8 December 1969 on control of Potato Cyst Eelworm (69/465/EEC). *Official Journal of the European Communities, Number L 323/3. 24.12.60.*
- ANON. (1991). Quarantine Procedure No. 30. *Globodera pallida* and *G. rostochiensis*. Soil sampling methods. *EPPO Bulletin* 21, 233-240.
- ANON. (1996). Potato Variety Handbook, Including NIAB recommended list of Potatoes 1997. National Institute of Agricultural Botany, Cambridge, UK, 60 pp.

- ANON. (2000). Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of European Communities* 43 no L169, pp. 1-112.
- ANTONIOU, M. (1989). Arrested development in plant-parasitic nematodes. *Helminthological Abstracts (Series B)* 58, 1-19.
- ATIBALENTJA, N. & NOEL, G. (1999). *Pasteuria* sp.: a promising biological control agent of the soybean cyst nematode. Proceedings of the National Soybean Cyst Nematode Conference. 7-8 January 1999, Orlando, Florida, USA, p. 51.
- ATKINSON, H.J. & BALLANTYNE, A.J. (1977). Changes in the oxygen consumption of cysts of *Globodera rostochiensis* associated with the hatching of juveniles. *Annals of Applied Biology* 87, 109-166.
- ATKINSON, H.J., LILLEY, C.J., URWIN, P.E. & MCPHERSON, M.J. (1998). Engineering resistance in potato to potato cyst nematodes. In: *Potato Cyst Nematodes, Biology, Distribution and Control* (eds R.J. Marks and B.B. Brodie). CAB International, Wallingford, UK, pp. 209-236.
- ATKINSON, H.J., TAYLOR, J.D. & BALLANTYNE, A.J. (1980). The uptake of calcium prior to hatching of the second stage juveniles of *Globodera rostochiensis*. *Annals of Applied Biology* 94, 103-109.
- AYERS, R. & LANE, A. (1986). Potato nematodes. ADAS, Report P. 3061, 4 pp.
- BAKKER, K.R., TOWNSHEND, J.L., MICHELLE, R.E., NORTON, D.C., RUEHLE, J.L., BIRD, G.W., CHAPMAN, R.A., DUNN, R.A., OOSTENBRINK, M., POWELL, W.M., THOMASON, I.J., FISHER, K.D., SOUTHARDS, C.J., MAI, W.F., SMART, G.C., ROHDE, R.A., DICKSON, D.W., MALEK, R.B., GRIFFIN, G.D., TAYLOR, P.L., THOMAS, W.H. & MURAD, J.L. (1978). Determining nematode population responses to control agents. In: *Methods for Evaluating Plant Fungicides, Nematicides and Bactericides* (eds E.I. Zehr, G.W. Bird, K.D. Fisher, K.D. Hickey, F.H. Lewis, R.F. Line and G.W. Richard). American Phytopathological Society, St Paul, Minnesota, USA, pp. 114-125.

- BALDWIN, J.G. & MUNDO-OCAMPO, M. (1991). Heteroderinae, cyst and non-cyst-forming nematodes. In: *Manual of Agriculture Nematology* (ed. W.R. Nickle). Marcel Dekker Inc., New York, USA, pp. 275-362.
- BARROWS, J.B. & RONCADORI, R.W. (1977). Endomycorrhizal synthesis by *Gigaspora margarita* in poinsettia. *Mycologia* 69, 1173-1184.
- BATES, J.A., TAYLOR, E.J.A, GANS, P.T. & THOMAS, J.E. (2002). Determination of relative proportions of *Globodera* species in mixed populations of potato cyst nematodes using PCR product melting peak analysis. *Molecular Plant Pathology* 3, 153-161.
- BEHRENS, E. (1975). *Globodera* Skarbilovich, 1959. Eine selbständige Gattung in der Unterfamilie Heteroderinae Skarbilovich, 1947 (Nematoda: Heteroderidae). In: *Vortragstagung zu Aktuellen Problemen der Phytonematologie*. 29 May 1975, Rostock, DDR, pp. 12-26.
- BERT, W., KARSSSEN, G., VAN DRIESCHE, R. & GERAERT, E. (2002). The cellular structure of the female reproductive system within the Heteroderinae and Meloidogyninae (Nematoda). *Nematology* 4, 953-963.
- BEUKEMA, H.P. & VAN DER ZAAG, D.E. (1990). Introduction to Potato Production. Pudoc, Wageningen, The Netherlands, 208 pp.
- BIRCHFIELD, W. & ANTONOPOULOS, A.A. (1976). Scanning electron microscopic observations of *Duboscqia penetrans* parasitising root knot larvae. *Journal of Nematology* 8, 272-273.
- BIRD, A.F. & BIRD, J. (1991). The structure of nematodes. Academic Press, San Diego, California, USA, 316 pp.
- BLOK, V.C., MALLOCH, G., HARROWER, B.E., PHILLIPS, M.S. & VRAIN, T.C. (1998). Intraspecific variation in ribosomal DNA in populations of potato cyst nematode *Globodera pallida*. *Journal of Nematology* 30, 262-274.
- BOUGHER, N.L., GROVE, T.S. & MALAJCZUK, N. (1990). Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor* F. Muell.) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. *New Phytologist* 114, 77-85.

- BOYDE, A.E.W. (1941). Detection of death in larvae of potato root eelworm. *Nature* 148, 782-783.
- BRODIE, B.B. (1982). Possible use of potato as a trap crop for controlling *Globodera rostochiensis* populations. *Journal of Nematology* 14, 432.
- BRODIE, B.B. & MAI, W.F. (1989). Control of the golden nematode in the United States. *Annual Review of Phytopathology* 27, 443-461.
- BROMILOW, R.H., BAKER, R.J., FREEMAN, M.A.H. & GÖRÖG, K. (1980). The degradation of aldicarb and oxamyl in soil. *Pesticide Science* 11, 371-378.
- BROWN, E.B. (1969). Assessment of the damage caused to potatoes by potato cyst eelworm, *Heterodera rostochiensis* Woll. *Annals of Applied Biology* 63, 493-502.
- BRUNDRETT, M.C. (1991). Mycorrhizas in natural ecosystems. In: *Advances in Ecological Research* (eds A. Macfayden, M. Begon and A.H. Fitter), Vol. 21. Academic Press, London, UK, pp. 171-313.
- BRUNDRETT, M.C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytologist* 154, 275-304.
- BRUNDRETT, M.C., BOUGHER, N., DELL, B., GROVE, T. & MALAJCZUK, N. (1996). *Working with mycorrhizas in forestry and agriculture*. ACIAR Monograph 32, Australian Centre for International Agricultural Research, Canberra, Australia, 374 pp.
- BRUNDRETT, M.C., MELVILLE, L. & PETERSON, L. (1994). *Practical Methods in Mycorrhizal Research*. Mycologue Publications, Waterloo, Canada, 161 pp.
- BURROWS, P.R. & BOFFEY, S.A. (1986). A technique for the extraction and restriction endonuclease digestion of total DNA from *Globodera rostochiensis* and *Globodera pallida* second-stage juveniles. *Revue de Nématologie* 9, 199-200.
- BYRNE, J. (1997). *Comparative hatching behaviour of Globodera rostochiensis and G. pallida*. Ph.D. Thesis, The National University of Ireland, Cork, Ireland, 302 pp.

- BYRNE, J., MAHER, N. & JONES, P.W. (2001). Comparative responses of *Globodera rostochiensis* and *G. pallida* to hatching chemicals. *Journal of Nematology* 33, 195-202.
- BYRNE, J., TWOMEY, U., MAHER, N., DEVINE K.J. & JONES P.W. (1998). Detection of hatching inhibitors and hatching factor stimulants for golden potato cyst nematode, *Globodera rostochiensis*, in potato root leachate. *Annals of Applied Biology* 132, 463-472.
- CABI (2000). Crop Protection Compendium. Global Module, 2<sup>nd</sup> edition, CD-ROM, CAB International, Wallingford, UK.
- CARROLL, M. (1995). *Characterisation of rhizosphere bacterial isolates which affect the hatch of potato cyst nematodes*. M.Sc. Thesis, The National University of Ireland, Cork, Ireland, 70 pp.
- CHEN, Z.X. & DICKSON, D.W. (1998). Review of *Pasteuria penetrans*: biology, ecology, and biological control potential. *Journal of Nematology* 30, 313-340.
- CHITWOOD, B.G. & CHITWOOD, M.B. (1950). Introduction to Nematology. University Park Press, Baltimore, USA, 334 pp.
- CLARKE, A.J. & HENNESSY, J. (1976). The distribution of carbohydrates in cysts of *Heterodera rostochiensis*. *Nematologica* 22, 190-195.
- CLARKE, A.J. & HENNESSY, J. (1987). Hatching agents as stimulants of movement of *Globodera rostochiensis* juveniles. *Revue de Nématologie* 10, 471-476.
- CLARKE, A.J. & PERRY, R.N., (1985). Egg-shell calcium and the hatching of *Globodera rostochiensis*. *International Journal for Parasitology* 15, 511-516.
- CLARKE, A.J., PERRY, R.N. & HENNESSY, J. (1978). Osmotic stress and the hatching of *Globodera rostochiensis*. *Nematologica* 24, 384-392.
- COLE, C.S. & HOWARD, H.W. (1962). Further results from a field experiment on the effect of growing resistant potatoes on a potato root eelworm (*Heterodera rostochiensis*) population. *Nematologica* 7, 57-61.



- COOMANS, A. (1975). Nematoda as a phylum. In: *Nematode Vectors of Plant Viruses* (eds F. Lamberti, C.E. Taylor and J.W. Seinhorst). Plenum Press, London and New York, 460 pp.
- COOPER, B.A. (1953). Eelworm problems in North Fenland with special reference to crop rotation. *Horticultural Education Association Annual Report* 106-115.
- CORBETT, D.C.M. & HIDE, G.A. (1971). Interactions between *Heterodera rostochiensis* Woll. and *Verticillium dahliae* Kleb. on potatoes and the effect of CCC on both. *Annals of Applied Biology* 68, 369-374.
- CRUMP, D.H. (1989). Interaction of cyst nematodes with their natural antagonists. *Aspects of Applied Biology* 22, 135-140.
- DEHNE, H.W. (1982). Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology* 72, 1115-1119.
- DEN NIJS, L.J.M.F & LOCK, C.A.M. (1992). Differential hatching of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* in root diffusates and water of differing ionic composition. *Netherlands Journal of Plant Pathology* 98, 117-128.
- DEN OUDEN, H. (1960). Periodicity in spontaneous hatching of *Heterodera rostochiensis* in soil. *Nematologica* 2, 101-105.
- DEN OUDEN, H. (1974). Aframe van de Bevolkings-dichtheid van hot aardappelcystenaaltje bij teelt van resistente aardappelen en bij braak. *Jaarverslag 1973 Instituut voor Plantenziektenkundig Onderzoek*, Wageningen, The Netherlands, 101.
- DEVINE, K.J. (1994). *Studies on the potato cyst nematode hatching factors of potato (Solanum tuberosum L.)*. M.Sc. Thesis, The National University of Ireland, Cork, Ireland, 194 pp.
- DEVINE, K.J. (2000). *Studies on hatching factors and potato cyst nematodes*. Ph.D. Thesis, The National University of Ireland, Cork, Ireland, 254 pp.

- DEVINE, K.J., BYRNE, J. & JONES, P.W. (2001). *In vitro* studies on the relative availability and mobility in soil of natural hatching factors for the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*. *Nematology* 3, 75-83.
- DEVINE, K.J., BYRNE, J., MAHER, N. & JONES, P.W. (1996). Resolution of natural hatching factors for the golden potato cyst nematode, *Globodera rostochiensis*. *Annals of Applied Biology* 129, 323-334.
- DEVINE, K.J., DUNNE, C., O'GARA, F. & JONES, P.W. (1999). The influence of in-egg mortality and spontaneous hatching on the decline of *Globodera rostochiensis* during crop rotation in the absence of the host potato crop in the field. *Nematology* 6, 637-645.
- DEVINE, K.J. & JONES, P.W. (2000). Response of *Globodera rostochiensis* to exogenously applied hatching factors in soil. *Annals of Applied Biology* 137, 21-29.
- DOLIWA, U. (1956). Experimentelle Untersuchungen an Kartoffelnematoden (*Heterodera rostochiensis* Wollenweber). *Wissenschaftliche Zeitschrift der Universität Rostock* 5, 133-149.
- DONCASTER, C.C. (1953). A study of host parasite relationships. The potato-root eelworm, *Heterodera rostochiensis* in black nightshade, *Solanum nigrum* and tomato. *Journal of Helminthology* 27, 1-8.
- DONCASTER, C.C & SEYMOUR, M.K. (1973). Exploration and selection of penetration site by *Tylenchida*. *Nematologica* 19, 137-145.
- DONCASTER, C.C & SHEPHERD, A.M. (1967). The behaviour of second-stage *Heterodera rostochiensis* larvae leading to their emergence from the egg. *Nematologica* 13, 476-478.
- ELLENBY, C. (1954). Tuber forming species and varieties of the genus *Solanum* tested for resistance to the potato root eelworm *Heterodera rostochiensis* Wollenweber. *Euphytica* 3, 195-202.
- ELLENBY, C. (1968). Desiccation survival in the plant parasitic nematodes *Heterodera rostochiensis* Wollenweber and *Ditylenchus dipsaci* (Kuhn) Filipjev. *Proceedings of the Royal Society of London, B.* 169, 203-213.

- ELLENBY, C. & PERRY, R.N. (1976). The influence of the hatching factor on the water uptake of the second stage larva of the potato cyst nematode *Heterodera rostochiensis*. *Journal of Experimental Biology* 64, 141-147.
- ELLENBY, C. & SMITH, L. (1967). Influence of temperature of polyphenol oxidase in three species of *Heterodera*. *Parasitology* 56, 165-170.
- ELLIOTT, M.J., PHILLIPS, M.S., TRUDGILL, D.L. & MCNICOL, J.W. (2000). The scope and limitations of a computer programme for potato cyst nematode management. *Aspects of Applied Biology* 59, 85-90.
- ELLIS, D. (1998). Soil's hidden heroes. *American Gardener* 77, 9-13.
- EPPO (2000). European and Mediterranean Plant Protection Organization. EPPO. Plant Quarantine Retrieval System. PQR. Version (3.10).
- EPPO/CABI (1997). *Globodera rostochiensis* and *Globodera pallida* Data Sheets on Quarantine Pests. In: *Quarantine Pests for Europe* (eds I.M. Smith, D.G. McNamara, P.R. Scott and M. Holderness). CAB International, Wallingford, UK, pp. 601-606.
- EVANS, K. (1970). Longevity of males and fertilisation of females of *Heterodera rostochiensis*. *Nematologica* 16, 369-374.
- EVANS, K. (1982). Effects of host variety, photoperiod and chemical treatments on hatching of *Globodera rostochiensis*. *Journal of Nematology* 14, 203-207.
- EVANS, K. (1983). Hatching of potato cyst nematodes in root diffusates collected from twenty-five potato cultivars. *Crop Protection* 2, 97-103.
- EVANS, K. (1993). New approaches for potato cyst nematode management. *Nematropica* 23, 221-231.
- EVANS, K. & BRODIE, B.B. (1980). The origin and distribution of the golden nematode and its potential in the USA. *American Potato Journal* 57, 70-89.
- EVANS, K. & HAYDOCK, P.P.J. (1990). A review of tolerance by potato plants of cyst nematode attack, with consideration of what factors may confer tolerance and methods of assaying and improving it in crops. *Annals of Applied Biology* 117, 703-740.

- EVANS, K. & HAYDOCK, P.P.J. (2000). Potato cyst nematode management - present and future. *Aspects of Applied Biology* 59, 91-97.
- EVANS, K. & PERRY, R.N. (1976). Survival strategies in nematodes. In: *The Organisation of Nematodes* (ed. N.A. Croll). Academic Press, London, UK, pp. 383-424.
- FENNER, L.M. (1962). Determination of nematode mortality. *Plant Disease Reporter* 46, 383.
- FENWICK, D.W. (1940). Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of Helminthology* 18, 155-172.
- FENWICK, D.W. & FRANKLIN, M.T. (1951). Further studies on the identification of *Heterodera* species by larval length. Estimation of the length parameters for eight species and varieties. *Journal of Helminthology* 25, 57-76.
- FENWICK, D.W. & WIDDOWSON, E. (1958). The conduct of hatching tests on cysts of the potato-root eelworm *Heterodera rostochiensis* (Woll.). *Journal of Helminthology* 32, 125-134.
- FLEMING, C.C. & MARKS, R.J. (1982). A method for quantitative estimation of *Globodera rostochiensis* and *G. pallida* in mixed species samples. *Record of Agricultural Research of the Department of Agriculture for Northern Ireland* 30, 67-70.
- FLEMING, C.C. & MARKS, R.J. (1983). The identification of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* by isoelectric focusing of proteins on polyacrylamide gels. *Annals of Applied Biology* 103, 277-281.
- FOLKERTSMA, R.T., VAN DER VOORT, J.N.A.M.R., VAN GENT PELZER, M.P.E., DE GROOT, K.E., VAN DEN BOS, W.J.R., SCHOTS, A., BAKKER, J. & GOMMERS, F.J. (1994). Interspecific and intraspecific variation between populations of *Globodera rostochiensis* and *Globodera pallida* revealed by random amplified polymorphic DNA. *Phytopathology* 84, 807-811.
- FORREST, J.M.S. & PERRY, R.N. (1980). Hatching of *Globodera pallida* eggs after brief exposure to potato root diffusate. *Nematologica* 26, 130-132.

- FORREST, J.M.S. & ROBERTSON, W.M. (1986). Characterisation and localization of sacharides on the head region of four populations of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Journal of Nematology* 18, 23-25.
- FRANCO, J. (1986). Potato cyst nematode *Globodera* spp. *Technical Information Bulletin* 9, International Potato Centre, Lima, Peru, 19 pp.
- FRANCO, J. & EVANS, K. (1979). Effects of daylength on the multiplication of potato cyst-nematode (*Globodera* spp.) populations. *Nematologica* 25, 184-190.
- FUKUZAWA, A., FURUSAKI, A., IKURA, M. & MASAMUNE, R. (1985). Glycinoeclepins B and C, nortriterpenes related to glycinoeclepin A. *Tetrahedron Letters* 26, 5539-5542.
- GAO, L.-L., DELP, G. & SMITH, S.E. (2001). Colonisation patterns in a mycorrhiza-defective mutant tomato vary with different arbuscular-mycorrhizal fungi. *New Phytologist* 151, 477-491.
- GERSTL, Z. (1984). Adsorption, decomposition and movement of oxamyl in soil. *Pesticide Science* 15, 9-17.
- GIANINAZZI-PEARSON, V., GOLLOTTE, A., LHERMINIER, J., TISSERANT, B., FRANKEN, P., DUMAS-GAUDOT, E., LEMOINE, M.C., VAN TUINEN, D. & GIANINAZZI, S. (1995). Cellular and molecular approaches in the characterisation of symbiotic events in functional arbuscular mycorrhizal associations. *Canadian Journal of Botany* 73, 526-532.
- GIOVANNETTI, M. & MOSSE, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular infection in roots. *New Phytologist* 84, 489-500.
- GIOVANNETTI, M. & SBRANA, C. (1998). Meeting a non-host: the behaviour of AM fungi. *Mycorrhiza* 8, 123-130.
- GOLDEN, A.M. (1971). Classification of the genera and the higher categories of the order Tylenchida. In: *Plant Parasitic Nematodes* (eds B.M. Zuckerman, W.F. Mai and R.A. Rhode). Academic Press, New York, USA, pp. 191-232.

- GOLDEN, A.M. (1986). Morphology and identification of cyst nematodes. In: *Cyst Nematodes* (eds F. Lamberti and C.E. Taylor). Plenum Press, London and New York, pp. 23-46.
- GOODEY, J.B., FRANKLIN, M.T. & HOOPER, D.J. (1965). *Nematode Parasites of Plants Catalogued under their Hosts*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, 214 pp.
- GRAY, N.F. (1988). Fungi attacking vermiform nematodes. In: *Diseases of Nematodes* (eds G.O.J. Poinar and H. Jansson). CRC Press, Boca Raton, Florida, USA, Vol. 2, pp. 3-38.
- GRECO N. (1988). Potato cyst nematodes: *Globodera rostochiensis* and *G. pallida*. *Nematology Circular No. 149*, Hawaii Department of Agriculture and Consumer Services.
- GRECO, N. (1993). Nematode problems affecting potato production in subtropical climates. *Nematropica* 23, 213-220.
- GRECO, N., DI VITO, M., BRANDONISIO, A., GIORDANO, I. & DE MARINIS, G. (1982). The effect of *Globodera pallida* and *G. rostochiensis* on potato yield. *Nematologica* 28, 379-386.
- GREEN, C.D. (1980). Nematode sex attractants. *Helminthological Abstracts (Series A)* 49, 327.
- GREINER, E., BOSSIS, M., FOUVILLE, D., RENAULT, L. & MUGNIÉRY, D. (2001). Molecular approaches to the taxonomic position of Peruvian potato cyst nematodes and gene pool similarities in indigenous and imported populations of *Globodera*. *Heredity* 86, 277-290.
- GROVE, I.G. & HAYDOCK, P.P.J. (2000). Toxicity of 1,3-dichloropropene to the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Aspects of Applied Biology* 59, 103-108.
- GROVE, I.G., HAYDOCK, P.P.J., EVANS, K. & LEWIS, D.J. (1999). Basal fertiliser application method, tuber initiation nitrogen, foliar NPK and the tolerance of potatoes to infection by the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Annals of Applied Biology* 134, 205-214.

- GUILE, C.T. (1970). Further observations on cyst colour changes in potato cyst nematode pathotypes. *Plant Pathology* 19, 1-6.
- HABTE, M., FOX, R.L., AZIZ, T. & EL-SWAIFY, S.A. (1988). Interaction of vesicular-arbuscular mycorrhizal fungi with erosion in an oxisol. *Applied and Environmental Microbiology* 54, 945-950.
- HAGUE, N.G.M & GOWEN, S.R. (1987). Chemical control of nematodes. In: *Principles and Practice of Nematode Control in Crops* (eds R.H. Brown and B.R. Kerry). Academic Press, New York, USA, pp. 131-173.
- HAGUE, N.G.M. & PAIN, B.F. (1970). Some observations on the effect of 'Temik' on the potato cyst-eelworm, *Heterodera rostochiensis* Woll. *Plant Pathology* 19, 69-71.
- HANCOCK, M. (1988). The management of potato cyst nematodes in the UK potato crops. *Aspects of Applied Biology* 17, 29-36.
- HAWKES, J.G. (1990). The Potato, Evolution, Biodiversity and Genetic Resources. Belhaven Press, London, UK, 259 pp.
- HAYDOCK, P.P.J. & EVANS, K. (1998). Management of potato cyst nematodes in the UK: an integrated approach? *Outlook in Agriculture* 27, 253-260.
- HAYMAN, D.S. (1983). The physiology of vesicular-arbuscular endomycorrhizal symbiosis. *Canadian Journal of Botany* 61, 944-963.
- HESLING, J.J. (1978). Cyst nematode: morphology and identification of *Heterodera*, *Globodera* and *Punctodera*. In: *Plant Nematology* (ed. J.F. Southey). H.M.S.O., London, UK, pp. 125-155.
- HOCKLAND, S. (2002). Potato cyst nematodes - a technical overview for England and Wales. Central Science Laboratory, York, UK, 17 pp.
- HOCKLAND, S., PICKUP, J. & TURNER, S. (2000). Potato cyst nematode - a plant health perspective for Great Britain and Northern Ireland. *Aspects of Applied Biology* 59, 11-18.

- HOMEYER, B. (1953). Die Unterscheidung lebender und toter Stockälchen (*Ditylenchus dipsaci* Kühn) durch Fluorochromierung mit Akridin-Orange. *Nachrichtenbl Deutsch Pflanzenschutzd. Braunschweig* 5, 8-11.
- HOMINICK, W.M. (1979). Selection for hatching at low temperatures in *Globodera rostochiensis* by continuous cultivation of early potatoes. *Nematologica* 25, 322-332.
- HOMINICK, W.M. (1986). Photoperiod and diapause in the potato cyst nematode *Globodera rostochiensis*. *Nematologica* 32, 408-418.
- HOMINICK, W.M., FORREST, J.M.S. & EVANS, A.A.F. (1985). Diapause in *Globodera rostochiensis* and variability in hatching trials. *Nematologica* 31, 159-170.
- HOOKE, W.J. (1981). *Compendium of Potato Diseases*. American Phytopathological Society, St Paul, Minnesota, USA, 125 pp.
- HOOPE, D.J. (1973). *Viruses and Invertebrates*. North Holland Publishing Company, Amsterdam, The Netherlands, 285 pp.
- HOOPE, D.J. (1978). The structure and classification of nematodes. In: *Plant Nematology* (ed. J.F. Southey). H.M.S.O., London, UK, pp. 3-45.
- HUSSEY, R.S. & RONCATORI, R.W. (1982). Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. *Plant Disease* 16, 9-14.
- JANOS, D.P. (1980). Mycorrhizae influence tropical succession. *Biotropica* 12, 56-64.
- JARSTFER, A.G. & SYLVIA, D.M. (1993). Inoculum production and inoculation strategies for vesicular-arbuscular mycorrhizal fungi. In: *Soil Microbial Ecology Applications in Agriculture and Environmental Management* (ed. F.B. Jr Metting). Marcel Dekker Inc., New York, USA, pp. 349-377.
- JATALA, P. (1986). Biological control of plant-parasitic nematodes. *Annual Review of Phytopathology* 24, 453-489.
- JONES, F.G.W. (1970). The control of the potato cyst nematode. *Journal of the Royal Society of Arts*. March 1970, 179-196.



- JONES, F.G.W. (1973). Management of nematode populations in Great Britain. Proceedings of the Fourth Tall Timbers Conference on Ecological Animal Control by Habitat Management. Number 5. The Station Publishers, 1-2 March 1973, Tall Timbers Research Station, Tallahassee, Florida, USA, pp. 81-86.
- JONES, F.G.W. & GANDER, M.C. (1962). A bioassay for *Heterodera spp.* without counting cysts or larvae. *Nematologica* 8, 39-50.
- JONES, F.G.W. & JONES, M.G. (1984). Pests of field crops. Thomson Litho Ltd., Scotland, 500 pp.
- JONES, M.D., DURALL, D.M. & TINKER, P.B. (1990). Phosphorus relationships and production of extramatrical hyphae by two types of willow ectomycorrhizas at different soil phosphorus levels. *New Phytologist* 115, 259-267.
- JONES, P.W., TYLKA, G.L. & PERRY, R.N. (1998). Hatching. In: *The Physiology and Biochemistry of Free-Living and Plant-Parasitic Nematodes* (eds R.N. Perry and D.J. Wright). CAB International, Wallingford, UK, pp. 181-212.
- KERRY, B.R. (1987). Biological control. In: *Principles and Practice of Nematode Control in Crops* (eds R.H. Brown and B.R. Kerry). Academic Press, Sydney, Australia, 340 pp.
- KERRY, B.R. (1990). An assessment of progress towards microbial control of plant-parasitic nematodes. *Journal of Nematology* 22, 621-631.
- KOCH, M., TANAMI, Z., BODANI, H., WININGER, S. & KAPULNIK, Y. (1997). Field application of vesicular-arbuscular mycorrhizal fungi improved garlic yield in disinfected soil. *Mycorrhiza* 7, 47-50.
- KORMANIK, P.P. & MCGRAW, A.C. (1982). Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: *Methods and Principles of Mycorrhizal Research* (ed. N.C. Schenck). American Phytopathological Society Press, St. Paul, Minnesota, USA, pp. 37-45.
- KORT, J., ROSS, H., RUMPERHORST, H.J. & STONE A.R. (1977). An international scheme for identifying and classifying pathotypes of potato cyst nematodes *G. rostochiensis* and *G. pallida*. *Nematologica* 23, 333-339.

- LAMONDIA, J.A. & BRODIE, B.B. (1986). The effects of potato trap crops and fallow on decline of *Globodera rostochiensis*. *Annals of Applied Biology* 108, 347-352.
- LANE, A. & HOLLIDAY, J.M. (1974). Potato root eelworm pathotype competition (ENEZ). Report on Field Experiments. ADAS, Report East Midlands Region 115-116.
- LEE, C.C., GREEN, R.G. & APT, W.J. (1986). Transformation and adsorption of Fenamiphos, F. sulfoxide and F. sulfone in Molokai soil and simulated movement with irrigation. *Journal of Contaminant Hydrology* 1, 211-225.
- LEISTRA, M., BROMILOW, R.H. & BOESTEN, J.J.T.I. (1980). Measured and stimulated behaviour of oxamyl in fallow soils. *Pesticide Science* 11, 379-388.
- LINDERMAN, R.G. (1988). Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology* 78, 366-371.
- LUC, M., MAGGENTI, A.R. & FORTUNER, R. (1988). A reappraisal of Tylenchina (Nemata). 9. The family Heteroderidae Filip'ev & Schuurmans Stekhoven, 1941. *Revue de Nématologie* 11, 159-176.
- MAGGENTI, A.R. (1971). Nemic relationships and the origins of plant parasitic nematodes. In: *Plant Parasitic Nematodes* (eds B.M. Zuckerman, W.F. Mai and R.A. Rohde). Academic Press, London and New York, pp. 65-81.
- MAI, W.F. & LEAR, B. (1953). The golden nematode. *Cornell Extension Bulletin*, 870.32 (Reprint No. 718).
- MARK, G.L. & CASSELLS, A.C. (1996). Genotype-dependence in the interaction between *Glomus fistulosum*, *Phytophthora fragariae* and the wild strawberry (*Fragaria vesca*). *Plant and Soil* 185, 233-239.

- MARSHALL, J.W. (1984). *The comparative biology of Globodera rostochiensis Woll. and Globodera pallida Stone (Nematoda: Heteroderidae) in the South Island of New Zealand*. Ph.D. Thesis, University of Canterbury, New Zealand 127 pp.
- MARSHALL, J.W. (1993). Detecting the presence and distribution of *Globodera rostochiensis* and *G. pallida* mixed populations in New Zealand using DNA probes. *New Zealand Journal of Crop and Horticultural Science* 21, 219-223.
- MASAMUNE, T., ANETAI, M., TAKASUGI, M. & KATSUI, N. (1982). Isolation of a natural hatching stimulus, glycinoeclepin A, for the soybean cyst nematode. *Nature* 297, 495-496.
- MCARTHUR, D.A.J. & KNOWLES, N.R. (1993). Influence of species of vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition on growth, development, and mineral nutrition of potato (*Solanum tuberosum* L.). *Plant Physiology* 102, 771-782.
- MCSORLEY, R. (1987). Extraction of nematodes and sampling methods. In: *Principles and Practice of Nematode Control in Crops* (eds R.H. Brown and B.R. Kerry). Academic Press, New York, USA, pp. 13-47.
- MEADOWS, J., GILL, S.S. & BONE, L.W. (1989). Factors influencing the lethality of *Bacillus thuringiensis kurstaki* toxin for eggs and larvae of *Trichostrongylus colubriformis* (nematode). *Journal of Parasitology* 75, 191-194.
- MELILLO, M.T., BLEVE-ZACHEO, T. & ZACHEO, G. (1990). Ultrastructural response of potato roots susceptible to cyst nematode *Globodera pallida* pathotype Pa3. *Revue de Nématologie* 13, 17-28.
- MINNIS, S.T., HAYDOCK, P.P.J., IBRAHIM, S.K., GROVE, I.G., EVANS, K. & RUSSELL, M.A. (2002). Potato cyst nematodes in England and Wales - Occurrence and distribution. *Annals of Applied Biology* 140, 187-195.
- MORTON, J.B. & BENNY, G.L. (1990). Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order Glomales, two new suborders, Glominae and Gigasporinae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon* 37, 471-491.

- MUGNIERY, D. & BALANDRAS, C. (1984). Examen des possibilités d'eradication du nématode kyste *G. pallida* Stone. *Agronomie* 4, 773-778.
- MUGNIERY, D. & FAYET, G. (1984). Determination du sexe de *Globodera rostochiensis* Woll et influence des nouveaoux d'infestations sur la pénétration, le developpement et le sexe de cet nématode. *Revue de Nématologie* 7, 233-238.
- MULHOLLAND, V., CARDE., L., O'DONNELL, K.J., FLEMING, C.C. & POWERS, T.O. (1996). Use of the polymerase chain reaction to discriminate potato cyst nematode at the species level. In: *Diagnostics in Crop Protection* (ed. G. Marshall). British Crop Protection Council Symposium Proceedings No. 65. British Crop Protection Council, Farnham Royal, UK, pp. 247-252.
- MULVEY, R.H. & STONE, A.R. (1976). Description of *Punctodera matadorensis* (Nematoda:Heteroderidae) from Saskatchewan with lists of species and generic diagnoses of *Globodera* (n. rank), *Heterodera* and *Sarisodera*. *Canadian Journal of Zoology* 54, 772-785.
- NICOLSON, T.H. (1960). Mycorrhiza in Gramineae II. Development in habitats particularly sand dunes. *Transactions of the British Mycological Society* 43, 132-214.
- NIJBOER, H. & PARLEVLIET, J.E. (1990). Pathotype-specificity in potato cyst nematodes, a reconsideration. *Euphytica* 49, 39-47.
- NORDMEYER, D. (1990). The search for novel nematocidal compounds. In: *Nematology from Molecule to Ecosystem* (eds F.J. Gommers and P.W.T. Maas). Proceedings of the Second International Nematology Congress. European Society of Nematologists Inc., 11-17 August 1990, Veldhoven, The Netherlands, pp. 281-293.
- OGIGA, I.R. & ESTEY, R.H. (1975). The use of Meldolas blue and Nile Blue A, for distinguishing dead from living nematodes. *Nematologica* 20, 271-276.
- OHR, H.D., SIMS, J.J. & GRECH, N.M. (1996). Methyl iodide, an ozone-safe alternative to methyl bromide as a soil fumigant. *Plant Disease* 80, 731-735.

- OOSTENDORP, M. & SIKORA, R.A. (1989). Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugarbeet. *Revue de Nématologie* 12, 77-83.
- OYDVIN, J. & HAMMERAAS, B. (1973). Lethal low temperatures for the cysts content of the potato-root eelworms. *Nematologica* 19, 568-569.
- PALKOVICS, Á. (2003). Occurrence of *Globodera pallida* in Hungary. *EPPO Bulletin* 33, 375-377.
- PEMBERTON, L.M.S., TSAI, S.L., LOVELL, P.H. & HARRIS, P.J. (2001). Epidermal patterning in seedling roots of eudicotyledons. *Annals of Botany* 87, 649-654.
- PEOPLES, S.A., MADDY, K.T., CUSIK, W., JACKSON, T., COOPER, C. & FREDERICKSON, A.D. (1980). A study of samples of well water collected from selected areas in California to determine the presence of DBCP and certain other pesticide residues. *Bulletin of Environmental and Contaminant Toxicology* 24, 611-618.
- PERRY, R.N. (1989). Dormancy and hatching of nematode eggs. *Parasitology Today* 5, 337-383.
- PERRY, R.N. (1997). Plant signals in nematode hatching and attraction. In: *Cellular and Molecular Aspects of Plant-Nematode Interactions* (eds C. Fenoll, F.M.W. Grundler and S.A. Ohl). Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 38-50.
- PERRY, R.N. & AUMANN, J. (1998). Behaviour and sensory responses. In: *The Physiology and Biochemistry of Free-Living and Plant-Parasitic Nematodes* (eds R.N. Perry and D.J. Wright). CAB International, Wallingford, UK, pp. 75-93.
- PERRY, R.N. & BEANE, J. (1982). The effect of brief exposures to potato root diffusate on the hatching of *Globodera rostochiensis*. *Revue de Nématologie* 5, 221-224.
- PERRY, R.N. & FEIL, J. (1986). Observations on a novel hatching bioassay for *Globodera rostochiensis* using fluorescence microscopy. *Revue de Nématologie* 9, 280-282.

- PERRY, R.N. & WHARTON, D.A. (1985). Cold tolerance of hatched and unhatched second stage juveniles of the potato cyst-nematode *Globodera rostochiensis*. *International Journal for Parasitology* 15, 441-445.
- PERRY, R.N., ZUNKE, U. & WYSS, U. (1989). Observations on the response of the dorsal and subventral oesophageal glands of *Globodera rostochiensis* to hatching stimulation. *Revue de Nématologie* 12, 91-96.
- PHILLIPS, J.M. & HAYMAN, D.A. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55, 158-161.
- PHILLIPS, M.S. (1984). The effect of initial population density on the reproduction of *Globodera pallida* on partially resistant potato clones derived from *Solanum vernei*. *Nematologica* 30, 57-65.
- PINOCHET, J., CALVET, C., CAMPRUBÍ, A. & FERNÁNDEZ, C. (1996). Interactions between migratory endoparasitic nematodes and arbuscular mycorrhizal fungi in perennial crops: A review. *Plant and Soil* 185, 183-190.
- PROT, J.C. (1980). Migration of plant-parasitic nematodes towards plant roots. *Revue de Nématologie* 3, 305-318.
- QUARLES, W. (1999). Plant disease biocontrol and VAM fungi. *The IPM Practitioner* 21, 1-9.
- RACKE, J. & SIKORA, R.A. (1992). Influence of the plant health promoting rhizobacteria *Agrobacterium radiobacter* and *Bacillus sphaericus* on *Globodera pallida* root infection and subsequent plant growth. *Journal of Phytopathology* 134, 198-208.
- RAUSCH, C., DARAM, P., BRUNNER, S., JANSÁ, J., LALOÍ, M., LEGGEWIE, G., AMRHEIN, N. & BUCHER, M. (2001). A phosphate transporter expressed in arbuscule-containing cells in potato. *Nature* 414, 462-470.
- RAWSTHORNE, D. & BRODIE, B.B. (1986). Relationship between root growth of potato, root diffusate production, and hatching of *Globodera rostochiensis*. *Journal of Nematology* 18, 379-384.

- RICE, S.L., LEADBEATER, B.S.C & STONE, A.R. (1985). Changes in cell structure in roots of resistant potatoes parasitised by potato cyst nematode. 1. Potatoes with resistant gene H<sub>1</sub> derived from *Solanum tuberosum* spp. *andigena*. *Physiological Plant Pathology* 27, 219-234.
- RIDING, A.E. & PARKER, W.E. (2000). The spatial distribution of potato cyst nematode populations: implications for reliable sampling strategies. *Aspects of Applied Biology* 59, 121-126.
- ROBINSON, M.P., ATKINSON, H.J. & PERRY, R.N. (1985). The effect of delayed emergence on infectivity of juveniles of the potato cyst nematode *Globodera rostochiensis*. *Nematologica* 31, 171-178.
- ROBINSON, M.P., ATKINSON, H.J. & PERRY, R.N. (1987). The influence of soil moisture and storage time on mobility, infectivity and lipid utilization of the second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Revue de Nématologie* 10, 343-348.
- ROLFE, S. (2003). Domestication of plants. In: *APS 206 Biotechnology and Food Module*. Department of Animal and Plant Sciences, University of Sheffield, 5 pp.
- ROSS, H. (1986). Potato breeding problems and perspectives. In: *Advances in Plant Breeding* (eds W. Horn and G. Robbelen). Verlag Paul Parey, Berlin/Hamburg, Germany, pp. 132.
- RYAN, N.A. (2003). *The effects of soil microorganisms on the hatch of potato cyst nematodes*. Ph.D. Thesis, The National University of Ireland, Cork, Ireland, 264 pp.
- RYAN, N.A., DUFFY, E.M., CASSELLS, A.C. & JONES, P.W. (2000). The effect of mycorrhizal fungi on the hatch of potato cyst nematodes. *Applied Soil Ecology* 15, 223-240.
- SAHAY, N.S., SINGH, S.A. & VARMA, A. (1998). Trends in endomycorrhizal research. *Indian Journal of Experimental Biology* 36, 1069-1086.
- SAYRE, R.M. & HWANG, S.W. (1975). Freezing and storing *Ditylenchus dipsaci* in liquid nitrogen. *Journal of Nematology* 7, 199-202.

- SAYRE, R.M., WERGIN, W.P., SCHMIDT, J.M. & STARR, M.P. (1991). *Pasteuria nishizawae* sp. nov., a mycelial and endospore-forming bacterium parasitic on cyst nematodes of genera *Heterodera* and *Globodera*. *Research in Microbiology* 142, 551-564.
- SCHOLTE, K. (1999). Control of potato cyst nematodes (*Globodera* spp.) using non-tuber bearing Solanaceae as trap crops. Proceedings of the 14<sup>th</sup> Triennial Conference of the European Association for Potato Research. 2-7 May 1999, Sorrento, Italy, pp. 319-320.
- SCHWEIGER, P.F., ROBSON, A.D. & BARROW, N.J. (1995). Root hair length determines beneficial effect of a *Glomus* species on shoot growth of some pasture species. *New Phytologist* 131, 247-254.
- SEINHORST, J.W. (1965). The relationship between nematode density and damage to plants. *Nematologica* 11, 137-154.
- SEINHORST, J.W. (1992). The relation between rate of reproduction and average number of eggs per cyst in *Globodera rostochiensis* and *G. pallida*. *Nematologica* 38, 335-352.
- SHEPHERD, A.M. (1962). New Blue R. A stain that differentiates between living and dead nematodes. *Nematologica* 8, 201-208.
- SHEPHERD, A.M. (1986). Extraction and estimation of cyst nematodes. In: *Laboratory Methods for Work with Plant and Soil Nematodes* (ed J.F. Southey). Reference book 402, H.M.S.O., London, UK, pp. 31-50.
- SHIELDS, R., FLEMING, C.C. & STRATFORD, R. (1996). Identification of potato cyst nematodes using the polymerase chain reaction. *Fundamental and Applied Nematology* 19, 167-173.
- SIDDIQUI, Z.A. & MAHMOOD, I. (1995). Role of plant symbionts in nematode management: A review. *Bioresource Technology* 54, 217-226.
- SIKORA, R.A. (1992). Management of the soil antagonistic potential in agroecosystems for nematode control. In: *Nematology from Molecule to Ecosystem* (eds F.J. Gommers and P.W.T. Maas). Proceedings of the Second International



- Nematology Congress. European Society of Nematologists Inc., 11-17 August 1990, Veldhoven, The Netherlands, pp. 249-256.
- SKENE, K.R. (1998). Cluster roots: some ecological considerations. *Journal of Ecology* 86, 1060-1064.
- SMART, G., STOKKERMANS, J.P.W.G., YAN, Y., DE BOER, J.M., BAUM, T.J., WANG, X., HUSSEY, R.S., GOMMERS, F.J., HENRISSAT, B., DAVIS, E.L., HELDER, J., SCHOTS, A. & BAKKER, J. (1998). Endogenous cellulase in animals: isolation of beta 1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. *Proceedings of the National Academy of Sciences of the United States of America* 95, 4906-4911.
- SMELT, J.H., DEKKER, A. & LEISTRA, M. (1979). Effect of soil moisture condition on the conversion rate of oxamyl. *Netherlands Journal of Agricultural Science* 27, 191-198.
- SMELT, J.H. & LEISTRA, M. (1992). Availability, movement and (accelerated) transformation to soil-applied nematicides. In: *Nematology from Molecule to Ecosystem* (eds F.J. Gommers and P.W.T. Maas). Proceedings of the Second International Nematology Congress. European Society of Nematologists Inc., 11-17 August 1990, Veldhoven, The Netherlands, pp. 266-280.
- SMELT, J.H., SCHUT, C.J., DEKKER, A. & LEISTRA, M. (1981). Movement and conversion of aldicarb and its oxidation products in potato fields. *Netherlands Journal of Plant Pathology* 87, 177-191.
- SMELT, J.H., VAN DE PEPPEL-GROEN, A.E., VAN DER PAS, L.J.T. & DIJKSTERHUIS, A. (1996). Development and duration of accelerated degradation of nematicides in different soils. *Soil Biology and Biochemistry* 28, 1757-1765.
- SMITH, G.S. (1987). Interactions of nematodes with mycorrhizal fungi. In: *Vistas on Nematology: A Commemoration of the Twenty-Fifth Anniversary of the Society of Nematologists* (eds J.A. Veech and D.W. Dickson). Society of Nematologists Inc., Hyattsville, Maryland, pp. 461-468.

- SMITH, J. & BROMILOW, R.H. (1977). Incorporation of granular nematicides in peat soils for control of potato-cyst nematode (*Heterodera rostochiensis*, Woll.). *Journal of Experimental Husbandry* 33, 98-111.
- SOUTHEY, J.F. (1970). Laboratory methods for work with plant and soil nematodes. *Technical Bulletin 2*. Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office, London, UK, 202 pp.
- SPAULL, A.M., TRUDGILL, D.L. & PHILLIPS, M.S. (1987). Behaviour of three new potato clones in integrated control systems for potato cyst nematodes, *Globodera* spp. *Proceedings of Crop Protection in Northern Britain*, pp. 196-201.
- STIRLING, G.R. (1988). Biological control of plant parasitic nematodes. In: *Diseases of Nematodes* (eds G.O. Poiner and H.B. Jansson). Vol. 2, CRC Press Inc., Boca Raton, Florida, USA, pp. 93-139.
- STIRLING, G.R. (1991). *Biological Control and Plant Parasitic Nematodes*. CAB International, Wallingford, UK, 275 pp.
- STONE, A.R. (1972). The round cyst species of *Heterodera* as a group. *Annals of Applied Biology* 71, 280-283.
- STONE, A.R. (1973a). *Heterodera pallida*. Commonwealth Institute of Helminthology Descriptions of Plant-Parasitic Nematodes, St. Albans, UK, Set 2, Nos. 16-17.
- STONE, A.R. (1973b). *Heterodera pallida* n. sp. (Nematoda: Heteroderidae), a second species of potato cyst nematode. *Nematologica* 18, 591-606.
- STONE, A.R. (1986). Taxonomy and phylogeny of cyst nematodes. In: *Cyst Nematodes* (eds F. Lamberti and C.E. Taylor). Plenum Press, London and New York, pp. 1-22.
- STONE, A.R., HOLLIDAY, J.M., MATHIAS, P.L. & PARROTT, D.M. (1986). A selective survey of potato cyst nematode pathotypes in Great Britain. *Plant Pathology* 35, 18-24.

- STOREY, G.W. (1984). The effect of oxamyl and the growing of susceptible and resistant potato cultivars on the population dynamics of *Globodera rostochiensis* throughout the soil profile. *Annals of Applied Biology* 104, 131-141.
- SYLVIA, D.M. (1998). Mycorrhizal symbioses. In: *Principles and Applications of Soil Microbiology* (eds D.M. Sylvia, J.J. Fuhrmann, P.G. Hartel and D.A. Zuberer). Prentice Hall, Upper Saddle River, New Jersey, USA, pp. 408-426.
- TAYLOR, C.E. (1986). Quarantine and legislation. In: *Cyst Nematodes* (eds F. Lamberti and C.E. Taylor). Plenum Press, London and New York, pp. 451-460.
- TINKER, P.B. (1984). The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. *Plant Soil* 76, 77-91.
- TRUDGILL, D.L. (1967). The effect of environment on sex determination in *Heterodera rostochiensis*. *Nematologica* 13, 263-272.
- TRUDGILL, D.L. (1985). Potato cyst nematodes: a critical review of the current pathotyping scheme. *European Plant Protection Organisation Bulletin* 15, 273-279.
- TRUDGILL, D.L. (1986). Yield losses caused by potato cyst nematode: a review of the current position in Britain and prospects for improvement. *Annals of Applied Biology* 108, 181-198.
- TRUDGILL, D.L. (2000). Management of plant parasitic nematodes. In: *Scottish Crop Research Institute Annual report 1999/2000* (eds W.H. MacFarlane Smith and T.D. Heilbronn), pp. 66-82.
- TRUDGILL, D.L. & CARPENTER, J.M. (1971). Disc electrophoresis of proteins of *Heterodera* species and pathotypes of *Heterodera rostochiensis*. *Annals of Applied Biology* 69, 35-41.
- TRUDGILL, D.L., EVANS, K. & FAULKNER, G. (1972). A fluidising column for extracting nematodes from soil. *Nematologica* 18, 469-475.
- TRUDGILL, D.L., EVANS, K. & PARROTT, D.M. (1975). Effects of potato cyst nematodes on potato plants. 1. Effects in a trial with irrigation and fumigation on

- the growth and nitrogen and potassium contents of a resistant and susceptible variety. *Nematologica* 21, 169-182.
- TRUDGILL, D.L., PHILLIPS, M.S. & ALPHEY, T.J.W. (1987). Integrated control of potato cyst nematode. *Outlook in Agriculture* 16, 167-172.
- TRUDGILL, D.L., PHILLIPS, M.S. & HACKETT, C.A. (1996). The basis of predictive modelling for estimating yield loss and planning potato cyst nematode management. *Pesticide Science* 47, 89-94.
- TSUTSUMI, M. (1976). Conditions for collecting the potato root diffusate and the influence on the natural hatching of potato cyst nematode. *Japanese Journal of Nematology* 6, 10-13.
- TURNER, S.J. (1985). Potato cyst nematode (eelworms) in Northern Ireland: Biology. *Agriculture Northern Ireland* 60, 230-232.
- TURNER, S.J. (1989). New sources of resistance to potato cyst nematodes in the Commonwealth potato collection. *Euphytica* 42, 145-153.
- TURNER, S.J. (1993). Soil sampling to detect potato cyst nematode (*Globodera* species). *Annals of Applied Biology* 123, 349-357.
- TURNER, S.J. (1996). Population decline of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) in field soils in Northern Ireland. *Annals of Applied Biology* 129, 315-322.
- TURNER, S.J. (1998). Sample preparation, soil extraction and laboratory facilities for the detection of potato cyst nematodes. In: *Potato Cyst Nematodes: Biology, Distribution and Control* (eds R.J. Marks and B.B. Brodie). CAB International, Wallingford, UK, pp. 75-90.
- TURNER, S.J., MARTIN, T.J.G., COPELAND, R.B., MARKS, R.J. & WATTS, P. (2000). Trap cropping of potato cyst nematodes using resistant Solanaceae potato clones. *Aspects of Applied Biology* 59, 59-66.
- TWOMEY, U. (1995). *Hatching chemicals involved in the interaction between potato cyst nematodes, host and non-host plants*. Ph.D. Thesis, The National University of Ireland, Cork, Ireland, 128 pp.

- TWOMEY, U., RAFTERY, T., DEVINE, K.J. & JONES, P.W. (1995). An improved procedure for assaying hatching activity towards *Globodera rostochiensis*. *Nematologica* 41, 258-268.
- URWIN, P.E., GREEN, J. & ATKINSON H.J. (2000). Resistance to *Globodera* spp. in transgenic *Solanum tuberosum* cv. Désirée that express proteinase inhibitors. *Aspects of Applied Biology* 59, 27-32.
- VAN DER VOORT, J.R., KANYUKA, K., VAN DER VOSSEN, E., BENDAHMANE, A., MOOIJMAN, P., KLEINLANKHORST, R., STIEKEMA, W., BAULCOMBE, D. & BAKKER, J. (1999). Tight physical linkage of the nematode resistance gene Gpa2 and the virus resistance gene Rx on a single segment introgressed from the wild species *Solanum tuberosum* subsp andigena CPC1673 into cultivated potato. *Molecular Plant Microbe Interactions* 12, 197-206.
- VESTBERG, M. & ESTAÚN, V. (1994). Micropropagated plants, an opportunity to positively manage mycorrhizal activities. In: *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems* (eds S. Gianinazzi and H. Schüepp). Birkhäuser Verlag, Basel, Switzerland, pp. 217-226.
- WEBLEY, D.P. & JONES, F.G.W. (1981). Observations on *Globodera pallida* and *G. rostochiensis* on early potatoes. *Plant Pathology* 30, 217-224.
- WESTCOTT, S.W. & KLUEPFEL, D.A. (1993). Inhibition of *Criconebella xenoplax* egg hatch by *Pseudomonas aurefaciens*. *Phytopathology* 83, 1245-1249.
- WHARTON, D.A. (1980). Nematode egg-shells. *Parasitology* 81, 447-463.
- WHITEHEAD, A.G. (1973). Control of cyst nematodes (*Heterodera* spp.) by organophosphates, oxycarbamates and soil fumigants. *Annals of Applied Biology* 75, 439-453.
- WHITEHEAD, A.G. (1977). Control of potato cyst nematode *Globodera rostochiensis* Ro-1, by picrolonic acid and trap crops. *Annals of Applied Biology* 87, 225-227.
- WHITEHEAD, A.G. (1986). Chemical and integrated control of cyst nematodes. In: *Cyst Nematodes* (eds F. Lamberti and C.E. Taylor). Plenum Press, London and New York, pp. 413-432.

- WHITEHEAD, A.G. (1991). The resistance of six potato cultivars to English populations of potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*. *Annals of Applied Biology* 118, 357-369.
- WHITEHEAD, A.G. (1992). Emergence of juvenile potato cyst nematodes *Globodera rostochiensis* and *G. pallida* and the control of *G. pallida*. *Annals of Applied Biology* 120, 471-486.
- WHITEHEAD, A.G. (1993). Integrated control of *G. pallida*. Final Report 1992. Potato Marketing Board, UK, 28 pp.
- WHITEHEAD, A.G. (1995). Decline of potato-cyst nematodes, *Globodera rostochiensis* and *G. pallida*, in spring barley microplots. *Plant Pathology* 44, 191-195.
- WHITEHEAD, A.G. (1998). Sedentary endoparasites of roots and tubers (I. *Globodera* and *Heterodera*). In: *Plant Nematode Control* (ed. A.G. Whitehead), CAB International, Wallingford, UK, pp. 146-208.
- WHITEHEAD, A.G. & TURNER, S.J. (1998). Management and regulatory control strategies for potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*). In: *Potato Cyst Nematodes, Biology, Distribution and Control* (eds R.J. Marks and B.B. Brodie). CAB International, Wallingford, UK, pp. 135-152.
- WILLIAMS, T.D. (1978). Cyst nematodes: Biology of *Heterodera* and *Globodera*. In: *Plant Nematology* (ed. J.F. Southey). H.M.S.O., London, UK, pp. 156-171.
- WINFIELD, A.L., ENFIELD, M.A. & FOREMAN, J.H. (1987). A column elutriator for extracting cyst nematodes and other small invertebrates from soil samples. *Annals of Applied Biology* 111, 213-231.
- WOLLENWEBER, H.W. (1923). Krankheiten und Schädigungen der Kartoffel. *Arbeiten des Forschungsinstitutes für Pflanzenzüchtung* 86, 148-157.
- WOODS, S.R. & HAYDOCK, P.P.J. (2000). Importance of correct granular nematicide application and incorporation for the control of potato cyst nematodes. *Aspects of Applied Biology* 59, 33-40.

- WOODS, S.R., HAYDOCK, P.P.J. & EDMUNDS, C. (1999). Mode of action of fosthiazate used for the control of the potato cyst nematode *Globodera pallida*. *Annals of Applied Biology* 135, 409-415.
- ZUCKERMAN, B.M. (1983). Hypothesis and possibilities of intervention in nematode chemoresponses. *Journal of Nematology* 15, 173-182.

## **Experimental Chapters**

**Chapter B.** Effects of a mixed-isolate mycorrhizal inoculum on the potato-potato cyst nematode interaction

**Chapter C.** Variation in plant growth and *in vitro* hatching activity towards potato cyst nematodes in the response of different potato cultivars to inoculation with arbuscular mycorrhizal fungus isolates

**Chapter D.** Studies on the effect of mycorrhization of potato roots on the hatching activity of potato root leachate towards the potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*

**Chapter E.** Interaction between arbuscular mycorrhizal fungi and the nematicide aldicarb on potato cyst nematode hatch, nematode development and yield of potatoes



## **Chapter B**

### **Effects of a mixed-isolate mycorrhizal inoculum on the potato-potato cyst nematode interaction**

By N A RYAN, T DELIOPOULOS, P JONES and P P J HAYDOCK

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The contribution of TD was the data in Table 3 and the relevant discussion

## Summary

Inoculation of microplants of potato cv. Golden Wonder with Vaminoc, a mycorrhizal inoculum of three arbuscular mycorrhizal fungi (*Glomus* spp.), resulted in an increase in in-sand hatch of *Globodera pallida*, but not *G. rostochiensis*, within 2 weeks. By this time, mycorrhized plants also supported a larger number of feeding nematodes of both PCN species (50% higher for *G. rostochiensis*) than did non-mycorrhized plants, with a higher proportion of the *G. pallida* population being fertilised females than for *G. rostochiensis*. After 12 weeks, the multiplication rate of *G. rostochiensis* on mycorrhized plants was significantly greater than on non-mycorrhized plants, whereas no such difference was observed for *G. pallida*.

The principal component of PCN multiplication affected by mycorrhization was increased cyst number per plant from 6 to 12 weeks. Over this period, there was no increase in cyst number per plant for either PCN species on non-mycorrhized plants, whereas the value increased on mycorrhized plants for both *G. rostochiensis* (by almost 200%) and *G. pallida* (57%). Mycorrhization resulted in significant increases in the root and shoot dry weights of plants grown in the absence of PCN. Although mycorrhized plants carried a larger PCN burden than non-mycorrhized plants when grown on PCN-infested medium, as a result of the increased PCN multiplication rate, they produced larger root systems than did non-mycorrhized plants, suggesting increased tolerance to PCN of the mycorrhized plants, particularly to *G. rostochiensis*. Of morphological characters investigated in the absence of PCN, only stem height (increased) was significantly affected by mycorrhization. Colonisation by mycorrhiza resulted in increased tuber yield both in the absence (significant increase) and presence (non significant) of PCN, as a result of increased

tuber number per plant. These results are discussed in the light of the possible use of AMF as part of an integrated PCN management plan.

**Key words:** Potato cyst nematode, *Globodera pallida*, *Globodera rostochiensis*, mycorrhiza, hatching, arbuscular mycorrhizal fungi, potatoes, integrated pest management

## Introduction

The potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) and *Globodera pallida* (Stone), are the dominant pests of potato crops in temperate regions (Fleming & Marks, 1988), causing losses in the EU estimated at £300M sterling each year. In the UK, for example, PCN are estimated to cause average annual yield losses of £43M sterling (Haydock & Evans, 1998), with *G. pallida* being the more important pest; Minnis *et al.* (2000) reported that 67% of the UK PCN populations were pure *G. pallida*, 8% pure *G. rostochiensis* and 25% mixed-species populations. These specialist monocyclic pests attack the roots of a small number of solanaceous species, including potato, tomato and aubergine, and their lifecycle is closely synchronised with that of the host plants, to maximise persistence and infectivity. Hatch of second-stage juveniles (J2s) from the 500 or more eggs held within each cyst (the remains of the mature egg-filled female) is induced by a family of at least nine host-specific hatching factors (Devine *et al.*, 1996; Jones, Tylka & Perry, 1998; Devine & Jones, 1999) present in potato root leachate (PRL).

Earlier studies had suggested that agents in the soil other than potato roots could influence PCN hatch (Ellenby & Smith, 1967; Perry, Hodges & Bean, 1981), with Fenwick (1956) and Tsutsumi (1976) suggesting that soil microbes caused

decreases or increases, respectively, in in-soil hatch of PCN. Tsutsumi (1976) hypothesised that seasonal flushes of hatching activity in soil in the absence of host plants was due to activities of soil micro-organisms. Devine *et al.* (1996) reported that potato plants grown under sterile conditions produced only a proportion of the HFs found in PRL from non-sterile-grown plants, suggesting that several of the HFs present in conventionally produced PRL may be microbial in origin. More direct evidence, that selected rhizobacteria affected hatching activity, was presented by Carroll (1995), who reported that selected isolates from the potato rhizosphere produced PCN species-specific HFs, while Cronin *et al.* (1997) demonstrated that the natural anti-fungal biocontrol chemical 2,4-diacetylphloroglucinol also stimulated hatch of *G. rostochiensis*. Furthermore, Racke & Sikora (1992) reported that free-living soil bacteria could influence potato-induced PCN hatch.

In addition to rhizoplane and rhizosphere bacteria, root-colonising micro-organisms also include mycorrhizal fungi. The arbuscular (endo-) mycorrhizal fungi (AMF) belong to the order Glomales and consist of six genera in three families, the Gigasporaceae, Glomaceae and Acaulosporaceae (Morton & Benny, 1990). They are all obligate symbionts and penetrate the plant root tissue intercellularly, eventually developing intra- and extracellular structures including arbuscules and vesicles, but they do not form a fungal root sheath (unlike the Ectomycorrhizae). Because AMF and root parasites occupy similar regions of the root system (Ingham, 1988), mycorrhizal fungi have been investigated as possible biocontrol agents (Gerdemann, 1964; Dehne, 1982; Vestberg, 1992; Mark & Cassells, 1996). AMF have been shown to reduce infestation, or damage by nematodes, as a result primarily of stimulatory effects on root growth (Hussey & Roncadori, 1982) although this study did not involve PCN. Ryan *et al.* (2000) demonstrated that inoculation of microplants of

potato cv. Golden Wonder with Vaminoc, a commercial mixture of three *Glomus* AMF species, increased the in-soil hatch of *G. pallida* but not *G. rostochiensis*. The study presented here was designed to investigate whether mycorrhizal colonisation affected other aspects of the PCN life-cycle.

## **Materials and Methods**

### **Plant material**

Potato plants used were microplants of cv. Golden Wonder, aseptically propagated *via* nodal culture (George, 1993). Plant tissue stocks were cultured on half-strength Murashige & Skoog medium (Murashige & Skoog, 1962): 2.21 g l<sup>-1</sup> Murashige & Skoog basal salts, 15 g l<sup>-1</sup> sucrose, 100 µg l<sup>-1</sup> kinetin, 200 µg l<sup>-1</sup> gibberellic acid, 6 g l<sup>-1</sup> agar, pH 5.8 in a growthroom (photosynthetic photon flux rate 300 µmol m<sup>-2</sup>s<sup>-1</sup>, under a 16-hour day at 22 ± 2°C, relative humidity day 55%, night 100%).

### ***In vivo* inoculation with AMF**

Microplants were inoculated at the time of transfer from culture vessels. Vaminoc (MicroBio Division, Agricultural Genetics Co. Ltd., Royston, Herts, UK) was placed (1g) into each planting hole at the time of transfer to a peat-based compost (Bord na Mona, Ireland) (Duffy, Hurley & Cassells, 2000). Plants were misted daily for one week whilst in a growth chamber. The study was conducted under glasshouse conditions of ambient temperature (minimum 16°C) and a 16-hour photoperiod, daylight supplemented by high-pressure sodium vapour lamps (400 W). Plants received weaning to glasshouse conditions for one week (i.e. plants were placed outdoors for increasing lengths of time each day to acclimatise them to

outdoor conditions) before experiments were set up. Uninoculated plants were produced in the same way, but without the inclusion of Vaminoc in the planting hole.

### **Determination of mycorrhizal colonisation**

Samples of root material, taken at 2, 6 and 12 weeks after planting, were cleared and stained with 0.05% (w/v) Trypan blue, and the percentage root colonised was determined using the grid line intersect method of Phillips & Hayman (1970).

### **Potato cyst nematodes**

Single-generation cysts of *G. rostochiensis* (pathotype Ro1) and *G. pallida* (Pa2/3) were used. Cysts were pre-soaked at 22°C on water-saturated discs of filter paper for one week prior to use.

### **Interaction of AMF, PCN and potato cv. Golden Wonder**

Vaminoc-inoculated and uninoculated microplants of potato cv. Golden Wonder were grown in 17.5 cm-diameter pots of washed quartz sand supplemented with slow-release solid fertiliser (3-month Osmocote; Grace Sierra, B.V. Herleen, The Netherlands) in the presence or absence of each PCN species. In order to aid their recovery at harvest, the cysts were placed in sealed muslin sachets (3 cm x 3 cm), each containing sixty pre-soaked cysts of one PCN species, a procedure used by Byrne (1997). Control pots (containing plants but no cysts and containing cysts but no plants) were also set up. The sachets were placed in each pot at a depth of 1–2 cm below the root system at planting. The pots were incubated in the glasshouse in a replicated randomised block design and covered with white oilcloth to reflect sunlight from the pots to minimise temperature elevation. Six replicate pots of each

of the eight treatments were harvested at each of the three harvest dates, 2, 6 and 12 weeks after planting.

### **Percentage hatch**

To determine percentage hatch after 2 weeks, the sachets from the relevant pots were harvested, three sub-samples of six cysts were recovered at random from each sachet and were immersed in 0.1% (w/v) aqueous Meldola's blue solution (Shepherd, 1962) for one week; samples had also been taken from the pre-soaked cysts prior to the experiment. The cysts were then washed and soaked in water overnight to remove excess stain. Following this, the cysts were placed in 200 µl of water and gently opened mechanically to free the eggs into the water. Three sub-samples (each 20 µl) were taken after the egg suspension had been thoroughly mixed using a vortex mixer. For each sub-sample, the numbers of stained unhatched eggs (non-viable eggs), unstained unhatched eggs (viable) and hatched eggs (each representing a hatched juvenile) were counted (only deeply stained eggs were classified as "stained") and the percentage hatch of the viable eggs was obtained using the following formula (Byrne, 1997):

$$\% \text{ viable egg hatch} = \frac{\text{number of hatched eggs}}{\text{number of hatched eggs} + \text{viable full eggs}} \times 100$$

### **Quantification of feeding juveniles**

The plants harvested 2 weeks after planting were used to quantify the number of juveniles feeding in the roots. The staining method followed the procedure of Byrd, Kirkpatrick & Barker (1983). The root systems were rinsed, cut into pieces (approximately 4 cm long) and soaked in 50 ml water and 20 ml 1.5% (w/v) sodium

hypochlorite solution for four minutes. The roots were then rinsed in water and left to soak for a further 15 minutes in fresh water to remove most of the bleach. Each root system was transferred into a beaker of 30 ml fresh water and containing three drops of acid-fuchsin stain (0.35 g acid fuchsin (w/v) in a 3:1 acetic acid: water ratio; Sigma-Aldrich, Dublin, Ireland). The beakers were microwaved at full power (650 W) for 1.5–2 minutes, during which the solution boiled for approximately 30 seconds; the nematodes stained red in the roots and were counted and expressed as number of feeding juveniles per root system.

### **Pf/Pi measurement**

The initial PCN population density ( $P_i$ ) was determined using the number of cysts per pot (60), the mean number of eggs and the percentage viability of the eggs, determined as described above. The corresponding values were determined from the cysts recovered after 6 and 12 weeks and used to calculate the final population size ( $P_f$ ); multiplication rate was presented as  $P_f/P_i$ .

### **Morphometric and physiological studies**

After 12 weeks, each plant was divided into root and shoot, each of which was dried at 60°C for 48 hours, and weighed. The total fresh weight of the tubers recovered from each pot was also determined after 12 weeks.

In a separate, more detailed experiment, morphometric and physiological studies were conducted on inoculated and uninoculated microplants grown in 20 cm diameter pots of peat-based potting compost, in the absence of PCN, with six replicates of each sample harvested 6 weeks after inoculation. For each plant, total leaf, root and shoot dry weight (after drying at 60°C for 48 hours), total leaf area



(using a 2-D image analysis system; DIAS 1.07, Delta-T Devices, Cambridge, UK) and mean specific leaf area (leaf area per unit leaf dry weight) were determined. Mean chlorophyll content (measured using a Minolta chlorophyll meter, measuring in arbitrary SPAD units) was determined on the youngest fully expanded leaf. Mean stem height and internode length was also assessed for each plant. After 12 weeks, tubers from a further six replicate plants were harvested for each treatment, and their numbers and fresh weights (average and total per plant) were determined.

### Data analysis

Statistical analysis on the data in Table 1, 2 and Fig. 2 was conducted using parametric interaction analysis of variance (ANOVA) using the statistical software Datadesk 5.0 (Data Description, Inc., Ithaca, New York). The data in Fig. 1 were analysed using non-parametric interaction ANOVA. The data in Table 3 was analysed using the unpaired t-test and the Mann-Whitney U-test.

## Results

### Root colonisation by AMF

Successful colonisation of the root systems of Vaminoc-inoculated potato plants was confirmed after 2 weeks. Mean ( $\pm$  SD) percentage root colonisation had reached  $81.8 \pm 8.6\%$  by 6 weeks, increasing significantly ( $P < 0.05$ ) to  $89.5 \pm 4.5\%$  after 12 weeks.

### Hatch

Under non-mycorrhizal plants, the percentage hatch of viable eggs of *G. pallida* after 2 weeks was significantly lower than that of *G. rostochiensis* (Table 1).

Vaminoc inoculation resulted in a marked ( $P < 0.10$ ) increase in hatch of *G. pallida* (from 62% to 71%) but not *G. rostochiensis* (from 86% to 88%), after 2 weeks. The ANOVA interaction table shows that there was a significant effect of the mycorrhiza on hatch ( $P < 0.001$ ), the significant (mycorrhiza x species) interaction ( $P < 0.01$ ) supports the observation that the hatch of the two PCN species responded differently to mycorrhizal inoculation. In the presence of AMF, however, the hatch of *G. pallida* was still significantly less than that of *G. rostochiensis* (Table 1). The spontaneous hatch of *G. rostochiensis*, i.e. in the absence of potato plants, was greater (though not significantly so) than that of *G. pallida* (Table 1).

**Table 1. Effect of mycorrhizal inoculation of potato cv. Golden Wonder on in-sand PCN hatch after 2 weeks**

Treatment	Percentage hatch		
	<i>Globodera pallida</i>	<i>Globodera rostochiensis</i>	
Non-mycorrhizal plants	62.62 b (b)	86.49 c (d)	
Mycorrhizal plants	71.39 b (c)	88.16 c (d)	
No plants	40.61 a (a)	46.39 a (a)	
Source of variation	df	F- ratio	Probability
Mycorrhizal treatment (M)	2	170.51	< 0.001
Species (S)	1	67.96	< 0.001
M x S interaction	2	6.02	< 0.01
Error	30		

Any two samples sharing a common letter were not significantly different ( $P < 0.05$ ) using the Tukey test (The 10% statistics are shown in brackets; any two samples sharing a common letter were not significantly different ( $P < 0.10$ ) using the Tukey test).

## Feeding

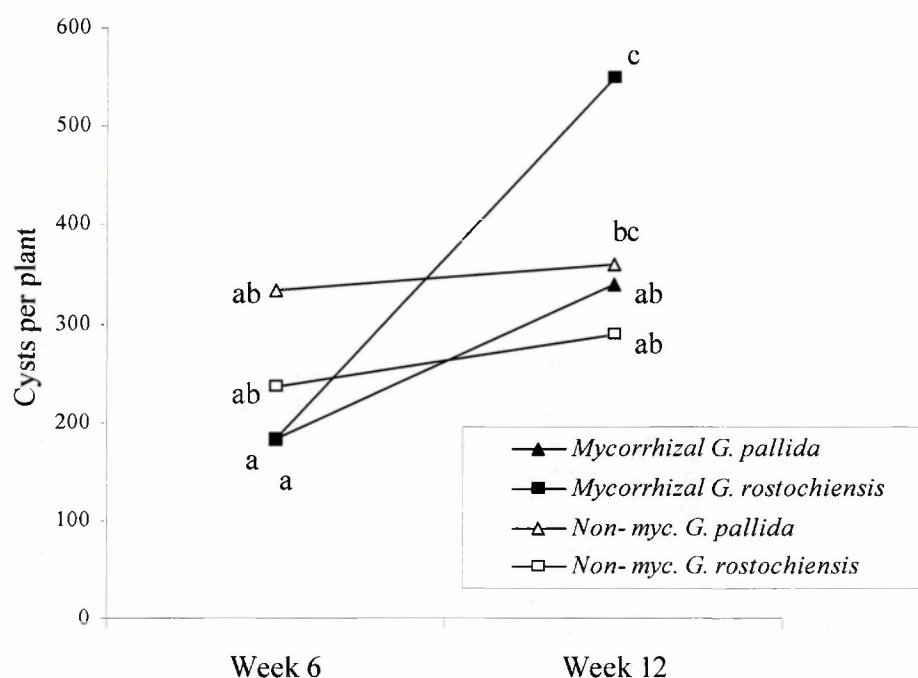
Greater numbers of feeding nematodes were observed per root system in the Vaminoc-inoculated plants (200 for *G. rostochiensis* and 196 for *G. pallida*) than in the uninoculated plants (134 for *G. rostochiensis*, 176 for *G. pallida*) 2 weeks after inoculation with PCN. At the same stage, a higher percentage of the nematodes feeding on the roots were fertilised females (visible as swollen pre-cysts) for *G. pallida* (29.6% in non-mycorrhizal, 27.0% in mycorrhizal plants) than for *G. rostochiensis* (21.6% and 19.0%, respectively). The differences (between PCN species or between mycorrhizal and non-mycorrhizal plants) were not significant.

## Cyst production

There was no significant increase in cyst production from 6 to 12 weeks in non-mycorrhizal plants inoculated with either PCN species (Fig. 1). In contrast, the number of *G. rostochiensis* cysts per plant increased significantly by 200% ( $P < 0.01$ ) whereas that of *G. pallida* increased by only 57% ( $P > 0.05$ ) in the presence of AMF (Fig. 1). Again there was a significant (species x mycorrhiza) interaction ( $P < 0.001$ ).

## Multiplication

The multiplication rate of *G. rostochiensis* and *G. pallida* on non-mycorrhizal plants was higher than on the Vaminoc-inoculated plants after 6 weeks (Table 2a), although the difference was not significant. This was the result of a greater number of viable eggs per cyst and cysts per plant in the non-mycorrhizal plants.



Source of variation	df	H-statistic	Probability
Sample	7	22.41	< 0.01
Mycorrhizal treatment	1	0.0043	> 0.05
Species	1	0.034	> 0.05
Time	1	13.17	< 0.001
Species x Mycorrhiza	1	22.38	< 0.001
Time x Mycorrhiza	1	9.24	< 0.01
Time x Species	1	9.21	< 0.01

**Fig. 1.** Effect of mycorrhizal inoculation on PCN cyst production on potato cv. Golden Wonder after 6 and 12 weeks. Any two samples sharing a common letter were not significantly different ( $P < 0.05$ ) using the Kruskal-Wallis test.

The multiplication rate of *G. rostochiensis* on Vaminoc-inoculated potato plants was significantly (approximately two-thirds;  $P < 0.01$ ) greater than that on non-mycorrhizal plants after 12 weeks, but there was no corresponding significant increase in the multiplication of *G. pallida* (Table 2b). For both PCN species, multiplication on mycorrhized plants (compared with non-mycorrhized plants) was associated with a lower percentage of viable eggs. The principal component of PCN multiplication affected by mycorrhization was the number of cysts per plant, with significantly more *G. rostochiensis* (but not *G. pallida*) cysts being produced on mycorrhized plants (Table 2b).

### **Morphometric and physiological studies**

In the absence of PCN, mycorrhization resulted in stimulatory effects on root dry weight (after 6 but not 12 weeks) and had a significant stimulatory effect on shoot dry weight (after 12 but not 6 weeks) of the potato plants (Fig. 2). Cultivation of mycorrhizal and non-mycorrhizal plants in the presence of PCN resulted in reductions in both root and shoot dry weight, especially after 12 weeks. At this date, there was a significant detrimental effect of *G. rostochiensis* with regard to both root and shoot dry weight of non-mycorrhizal plants, whereas *G. pallida* only significantly affected shoot dry weight (Fig. 2b). Mycorrhization resulted in (non-significant) increases in root and shoot dry weight in the presence of either *G. rostochiensis* or *G. pallida*, compared to non-mycorrhized plants (Fig.2). In the presence of *G. rostochiensis*, the root dry weights of the non-mycorrhizal plants were significantly less than that of the control plants, in the absence of PCN (Fig. 2a); however, inoculation with Vaminoc did not decrease the root dry weight significantly compared to the control, and the root dry weight of the *G. rostochiensis*-

**Table 2. Effect of arbuscular mycorrhizal fungi (AMF) colonisation on multiplication of the two PCN species on potato cv. Golden Wonder after a) 6 weeks and b) 12 weeks**

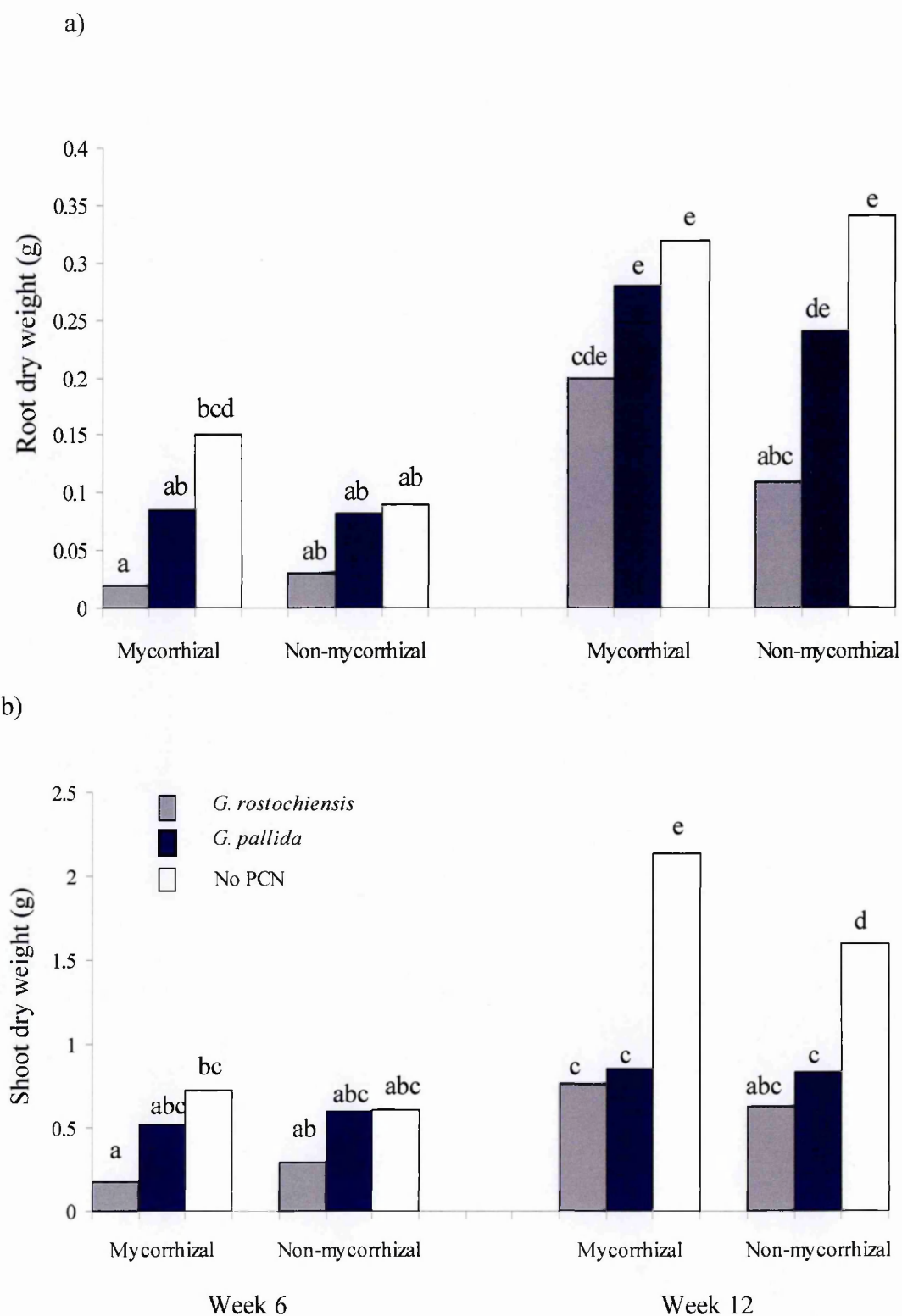
**a) 6 weeks**

Component	<i>G. rostochiensis</i>		<i>G. pallida</i>	
	- AMF	+ AMF	- AMF	+ AMF
Eggs per cyst	301 ab	254 a	329 ab	368 b
% viability	80.1 ab	75.5 a	88.0 b	77.9 a
Number of viable eggs per cyst	241 ab	192 a	290 b	287 b
Number of cysts per plant	235 a	184 a	353 a	183 a
Total number of eggs (Pf)	56635 ab	35328 a	102370 b	52521 ab
Multiplication rate (Pf / Pi)	2.29 a	1.43 a	2.93 a	1.50 a

**b) 12 weeks**

Component	<i>G. rostochiensis</i>		<i>G. pallida</i>	
	- AMF	+ AMF	- AMF	+ AMF
Eggs per cyst	960 b	948 b	749 a	879 ab
% viability	80.1 ab	75.5 a	88.0 b	77.9 a
Number of viable eggs per cyst	769 a	716 a	659 a	685 a
Number of cysts per plant	288 a	550 b	360 a	340 a
Total number of eggs (Pf)	221472 a	393800 b	237240 a	232900 a
Multiplication rate (Pf / Pi)	8.94 a	15.89 b	6.79 a	6.67 a

The multiplication rate of PCN was based on the number of eggs. The Pi for *G. rostochiensis* was 24,780 eggs (413 viable eggs per cyst x 60 cysts), the corresponding Pi for *G. pallida* was 34,920 eggs (582 viable eggs per cyst x 60 cysts). Any two samples in the same row sharing a common letter were not significantly different ( $P < 0.05$ ) using the Tukey test.



**Fig. 2.** Effect of mycorrhizal inoculation on (a) root and (b) shoot dry weights of potato cv. Golden Wonder after 6 and 12 weeks in the presence and absence of PCN. Any two samples sharing a common letter are not significantly different ( $P < 0.05$ ) using the Tukey test.

infected mycorrhized plants was significantly greater ( $P < 0.10$ ) than the *G. rostochiensis*-infested non-mycorrhized plants. In Figs 2a and 2b there was a significant effect on root and shoot dry weight by the mycorrhiza ( $P < 0.05$ , Table 4).

Similar trends for average tuber weight were obtained for mycorrhizal and non-mycorrhizal plants grown both in the absence of PCN (2.05 and 1.58 g, respectively) and in the presence of *G. pallida* (2.45 and 2.11 g, respectively). In the presence of *G. rostochiensis*, mycorrhizal plants produced much larger tubers (2.51 g) than did non-mycorrhizal plants (0.61 g), although the existence of plants which failed to tuberise in both samples meant that the difference was not statistically significant.

In a more detailed analysis (Table 3) of the effects of Vaminoc inoculation on morphological and physiological parameters of potato cv. Golden Wonder in the absence of PCN, significant differences between mycorrhized and non-mycorrhized plants were observed only for shoot height ( $P < 0.05$ ), where mycorrhizal plants were significantly taller, and total tuber fresh weight per plant ( $P < 0.001$ ), with the mycorrhizal plants producing the significantly greater fresh tuber yield (Table 3). The increase in stem height was associated with a similar increase in mean internode length, but not with an increase in stem weight (Table 3).



Table 4. Interaction ANOVA summary table for data in Fig. 2

**a) root dry weight**

Source of variation	df	F-statistic	Probability
PCN treatment	2	26.42	< 0.001
Time	1	141.79	< 0.001
Mycorrhizal treatment	1	4.44	< 0.05
Time x Mycorrhiza	1	1.17	> 0.05
Time x PCN treatment	2	2.25	> 0.05
Mycorrhiza x PCN treatment	2	0.57	> 0.05
Error	62		

**b) shoot dry weight**

Source of variation	df	F-statistic	Probability
PCN treatment	2	76.76	< 0.001
Time	1	140.38	< 0.001
Mycorrhizal treatment	1	3.79	< 0.10
Time x Mycorrhiza	1	5.90	< 0.05
Time x PCN treatment	2	26.87	< 0.001
Mycorrhiza x PCN treatment	2	4.71	< 0.05
Error	62		

**Table 3. Effects of mycorrhizal colonisation on morphological and physiological characters of potato cv. Golden Wonder after 6 weeks (mean  $\pm$  S.D.)**

Character	Minus-AMF	Plus-AMF	Analysis	<i>P</i>
Internode length (cm)	1.35 $\pm$ 0.43	1.56 $\pm$ 0.39	<i>t</i> = 0.89	> 0.05
Chlorophyll content (SPAD units)	48.62 $\pm$ 2.21	51.56 $\pm$ 3.09	<i>t</i> = 1.64	> 0.05
Specific leaf area (mg cm <sup>-2</sup> )	2.58 $\pm$ 0.10	2.59 $\pm$ 0.13	<i>t</i> = 0.15	> 0.05
Total leaf area* (cm <sup>2</sup> )	21.33 (454.9) $\pm$ 2.44	20.63 (425.6) $\pm$ 1.4	<i>t</i> = 0.61	> 0.05
Shoot height* (cm)	5.71 (32.60) $\pm$ 0.55	6.43 (41.34) $\pm$ 0.35	<i>t</i> = 2.71	< 0.05
Leaf dry weight (g)	1.20 $\pm$ 0.28	1.11 $\pm$ 0.13	<i>t</i> = 0.71	> 0.05
Number of leaves	85.33 $\pm$ 9.87	88.00 $\pm$ 14.79	U = 17.5	> 0.05
Shoot dry weight (g)	1.06 $\pm$ 0.26	1.04 $\pm$ 0.17	<i>t</i> = 0.15	> 0.05
Number of stems	7.67 $\pm$ 2.58	7.00 $\pm$ 1.26	<i>t</i> = 0.57	> 0.05
Root dry weight (g)	0.21 $\pm$ 0.04	0.21 $\pm$ 0.06	<i>t</i> = 0.01	> 0.05
Tubers fresh weight, per plant (g)	4.79 $\pm$ 0.70	7.49 $\pm$ 1.10	<i>t</i> = 5.06	< 0.001
Tubers fresh weight, average (g)	0.94 $\pm$ 0.35	0.86 $\pm$ 0.12	<i>t</i> = 1.57	> 0.05
Number of tubers	5.67 $\pm$ 1.97	8.33 $\pm$ 1.94	<i>t</i> = 2.81	< 0.05

Data analysis was conducted by unpaired t-test (statistic *t*) or Mann-Whitney U-test (statistic *U*). Asterisk denotes variable square root transformed prior to analysis. Back-transformed means are presented in parentheses.

## Discussion

The effect of AMF inoculation on PCN hatch, stimulating hatch of *G. pallida* but not *G. rostochiensis*, under glasshouse conditions, was similar to that reported for outdoor-grown plants by Ryan *et al.* (2000) although, in the latter case, the effect of AMF on *G. pallida* hatch was significant at 5% level whereas here it was significant at the 10% level. The behaviour of PCN under non-mycorrhizal plants resembled the situation reported previously from field studies, with *G. rostochiensis* hatching earlier than *G. pallida* under potato plants and in greater numbers than *G. pallida* in the absence of potato plants (“spontaneous hatch”) (Whitehead, 1992). These findings suggest that the results from glasshouse studies of PCN hatch under pot-grown plants can be extrapolated (albeit with caution) to the situation in the field.

The increased PCN hatch associated with mycorrhized plants could be due to the larger root systems (the principal site of leaching of hatching chemicals from the host plant) of mycorrhized plants (Fig. 2), but the PCN species-specificity of the effect of AMF colonisation on hatch suggests a more complex cause. Increased species-specific PCN hatch could result from altered levels of any one of three classes of natural hatching chemical (hatching factors, HFs, hatch inhibitors, HIs, or hatching factor stimulants, HSs; Byrne *et al.*, 1998), each of which is known to exhibit PCN species-selectivity and -specificity (Byrne *et al.*, 2001); further analysis of this phenomenon will require fractionation and comparison of hatching chemicals present in leachate collected from mycorrhizal and non-mycorrhizal plants. This would also help to determine whether increased *G. pallida* hatch was due to altered production of potato hatching chemicals or to the generation of novel chemicals associated with the mycorrhizal interaction.

The effect of mycorrhization on PCN hatch was specific to *G. pallida*, but the effects of AMF inoculation on the later stages of the PCN life cycle (establishment of feeding sites, multiplication) were largely restricted to *G. rostochiensis*. The significant increase in *G. rostochiensis* cyst numbers per root system in mycorrhized but not non-mycorrhized plants from week 6 to week 12 indicated that the period of cyst production in mycorrhized plants was longer than in non-mycorrhized plants; a smaller and non-significant effect was also observed for *G. pallida*. This effect on cyst production mirrored the greater numbers of *G. rostochiensis* and *G. pallida* nematodes feeding on root systems of mycorrhized as compared to non-mycorrhized plants after 2 weeks. At week 6, the multiplication rate was higher for both species on the non-mycorrhizal plants and, although there was a greater number of nematodes feeding on the mycorrhizal plants, the number of developing females on these plants was lower, suggesting that although more nematodes were present in the root system (possibly due to the larger root systems), fewer females were allowed to develop at this stage. Although fewer developing females were present at week 2 the period of cyst production for both PCN species under mycorrhizal plants was increased markedly.

This prolongation of the period of cyst production in mycorrhizal plants could be due to greater food supply to the nematodes, such as increases in the numbers of feeding sites on root systems of mycorrhized plants (e.g. as a result of increased root system size, or a change in root development, such as increased branching), resulting in larger proportions of females. Supporting this hypothesis, mycorrhizal plants (in the absence of PCN) produced a significantly larger root system after 6 (but not 12) weeks than the corresponding non-mycorrhizal plants (Fig. 2a). The consequence was a significant increase in the multiplication of *G. rostochiensis* in the presence of

the mycorrhizal fungus, as a result of an increase in cyst number. In a study of plant-parasitic nematodes which did not include PCN, Hussey & Roncadori (1982) reported reduced nematode infestation in plants inoculated with AMF, while Sikora (1981) found that potatoes colonised by the AMF *Glomus fasciculatum* exhibited a reduction in population size of *G. rostochiensis* compared to non-mycorrhized plants. These authors considered that these reductions in nematode population size could be the result of competition between nematodes and AMF for nutrients or habitable space within the root (Hussey & Roncadori, 1978).

Infestation with either PCN species resulted in reductions in root weight by week 6 in mycorrhizal but not non-mycorrhizal plants (Fig. 2), significantly so in the case of *G. rostochiensis*. Although this result suggested reduced tolerance to PCN attack in mycorrhizal compared to non-mycorrhizal plants, closer examination of the data showed that this effect was due to the greater root weight of the non-PCN-infested mycorrhizal plants compared to the corresponding non-mycorrhizal controls; the root dry weights of PCN-infested plants were similar for mycorrhizal and non-mycorrhizal plants. By 12 weeks, feeding and multiplication of either PCN species had resulted in reduced root weights of both mycorrhized and non-mycorrhized plants, but this decrease was significant only in the case of the *G. rostochiensis*-infested non-mycorrhizal plants. Interestingly, mycorrhized plants infested with either PCN species produced root systems larger (though not significantly so) than those of their non-mycorrhized counterparts; the lack of a similar effect on shoot weight meant that the mycorrhized plants exhibited an increased root: shoot ratio. The fact that this effect on the root system happened even with *G. rostochiensis*, where the mycorrhizal plants carried a 60% higher PCN burden than the non-mycorrhizal plants, suggests that mycorrhiza can increase potato crop tolerance in

PCN (particularly *G. rostochiensis*) by increasing the size of the root system, a phenomenon already noted with respect to other plant-nematode combinations (Hussey & Roncadori, 1982). This increased tolerance was the converse of the ability of the larger root system to support a larger PCN egg population, with a resulting increase in PCN (particularly *G. rostochiensis*) multiplication. The increased PCN tolerance was also manifested in the ability of mycorrhized plants to maintain high tuber weight in the face of PCN infestation.

The results of this study indicated that mycorrhization of potato plants could affect the potato-PCN interaction in several ways. The delayed early hatch of *G. pallida*, compared to *G. rostochiensis*, was proposed by Whitehead (1992) as a reason for poor control of this species in the field by granular nematicides, which would have degraded to a sub-optimal concentration by the time *G. pallida* had hatched. Acceleration of the early hatch of *G. pallida* by mycorrhization could improve nematicidal control of this PCN species. On the other hand, mycorrhization could increase the tolerance of a crop to PCN, particularly *G. rostochiensis*, albeit at the cost of increased PCN multiplication.

A pre-requisite of the use of AMF inoculation in any PCN control programme would be that mycorrhization would have no negative effects on potato plant growth, development or yield characteristics in the absence of PCN. The only morphological trait significantly affected by Vaminoc inoculation of potato cv. Golden Wonder was stem height (Table 3); the observations on mean internode length (increased in mycorrhized plants) and stem weight (unaffected) suggest that the increase in plant height could be associated with reduced stem thickness, which could be a detrimental character, if confirmed. Mycorrhization in the absence of PCN also resulted in a significant increase in tuber weight in cv. Golden Wonder (Table 3); this effect was

supported by the results from the PCN study, where, in all three mycorrhized/ non-mycorrhized comparisons (minus-PCN, plus-*G. rostochiensis*, plus-*G. pallida*), the mycorrhized plants produced higher tuber yields than did the non-mycorrhized plants; the low weights of tubers were associated with the use of pot-grown plants.

These studies on the effects of mycorrhization on the potato-PCN interaction need to be expanded to include more varieties (to determine whether the effects are variety-independent) and more AMF isolates, and to progress the research to the field situation. Clearly, a better understanding of the complexity of the potato-PCN-AMF-nematicide interaction and more research is needed before mycorrhization can be recommended as part of integrated pest management for PCN, but the initial results are promising.

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## References

- Byrd D, Kirkpatrick T, Barker K R. 1983. An improved technique for clearing and staining plant tissue for detection. *Journal of Nematology* **15**:142-143.
- Byrne J. 1997. *Comparative hatching behaviour of Globodera rostochiensis and G. pallida*. PhD thesis, The National University of Ireland, Cork, Ireland.

- Byrne J, Maher N J, Jones P W. 2001.** Comparative responses of *Globodera rostochiensis* and *G. pallida* to hatching chemicals. *Journal of Nematology* **33**: 195-202.
- Byrne J, Twomey U, Maher N, Devine K J, Jones P W. 1998.** Detection of hatching inhibitors and hatching factor stimulants for golden potato cyst nematode, *Globodera rostochiensis*, in potato root leachate. *Annals of Applied Biology* **132**:463-472.
- Carroll M. 1995.** *Characterisation of rhizosphere bacterial isolates which affect the hatch of potato cyst nematodes*. MSc thesis, The National University of Ireland, Cork, Ireland.
- Cronin D, Moenne-Loccoz Y, Fenton A, Dunne C C, Dowling D N, O’Gara F. 1997.** Role of 2,4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. *Applied and Environmental Microbiology* **63**:1357-1361.
- Dehne H W. 1982.** Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology* **72**:1115-1119.
- Devine K J, Byrne J, Maher N, Jones P W. 1996.** Resolution of natural hatching factors for the golden potato cyst nematode, *Globodera rostochiensis*. *Annals of Applied Biology* **129**:323-334.
- Devine K J, Jones P W. 1999.** Purification and partial characterisation of hatching factors for the potato cyst nematode *Globodera rostochiensis* from potato root leachate. *Nematology* **2**:231-236.
- Duffy E M, Hurley E M, Cassells A C. 2000.** Weaning performance of potato microplants following bacterization and mycorrhization. *Potato Research* **42**:521-527.



- Ellenby C, Smith L. 1967. Emergence of larvae from new cysts of the potato root eelworm, *Heterodera rostochiensis*. *Nematologica* **13**:272-278.
- Fenwick D W. 1956. The hatching of cyst-forming nematodes. *Report of Rothamsted Experimental Station for 1955*, pp. 202-209.
- Fleming C C, Marks R J. 1988. Biochemical systematics of potato cyst nematodes. In *Taxonomy in the 20<sup>th</sup> Century. Proceedings of the Royal Irish Academy*, pp. 1-13. Ed. C Moriarty. Dublin, Ireland: Royal Irish Academy.
- George E F. 1993. Plant propagation by tissue culture, Part 1. Second edition, Exegetics Ltd, England.
- Gerdemann J W. 1964. The effect of mycorrhiza on the growth of maize. *Mycologia* **54**:342-349.
- Haydock P P J, Evans K. 1998. Management of potato cyst nematodes in the UK: an integrated approach? *Outlook in Agriculture* **27**:253-260.
- Hussey R S, Roncadori R W. 1978. Interactions of *Pratylenchus brachyurus* and *Gigaspora margarita* on cotton. *Journal of Nematology* **10**:16-20.
- Hussey R S, Roncadori R W. 1982. Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. *Plant Disease* **16**:9-14.
- Ingham R W. 1988. Interactions between nematodes and vesicular-arbuscular mycorrhizae. *Agriculture, Ecosystems and Environment* **24**:169-182.
- Jones P W, Tylka G L, Perry R N. 1998. Hatching. In *The Physiology and Biochemistry of Free-living and Plant Parasitic Nematodes*, pp. 181-212. Eds R N Perry and D J Wright. Wallingford, UK: CAB International.
- Mark G L, Cassells A C. 1996. Genotype-dependence in the interaction between *Glomus fistulosum*, *Phytophthora fragariae* and the wild strawberry (*Fragaria vesca*). *Plant and Soil* **185**:233-239.

- Minnis S T, Haydock P P J, Ibrahim S K, Grove I G. 2000.** The occurrence and distribution of potato cyst nematodes in England and Wales. *Aspects of Applied Biology* **59** (*Potato Cyst Nematode Management*): 1-9.
- Morton J B, Benny G L. 1990.** Revised classification of arbuscular mycorrhizal fungi (Zygomycetes) with a new order, Glomales, two new sub-orders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an amendment of Glomaceae. *Mycotaxon* **37**:471-491.
- Murashige T, Skoog F. 1962.** A revised medium for rapid growth and biomass assays with tobacco tissue cultures. *Physiologia Plantarum* **15**:473-497.
- Perry R N, Hodges J A, Beane J. 1981.** Hatching of *Globodera rostochiensis* in response to potato root diffusate persisting in soil. *Nematologica* **27**:349-352.
- Phillips J M, Hayman D A. 1970.** Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**:158-161.
- Racke J, Sikora R A. 1992.** Influence of the plant health promoting rhizobacteria *Agrobacterium radiobacter* and *Bacillus sphaericus* on *Globodera pallida* root infection and subsequent plant growth. *Journal of Phytopathology* **134**:198-208.
- Ryan N A, Duffy E, Cassells A C, Jones P W. 2000.** The effect of mycorrhizal fungi on the hatch of potato cyst nematodes. *Applied Soil Ecology* **15**:233-240.
- Shepherd A M. 1962.** New Blue R, a stain that differentiates between living and dead nematodes. *Nematologica* **8**:201-208.
- Sikora R A. 1981.** Interactions between plant-parasitic nematodes, plant roots and vesicular-arbuscular mycorrhizae. In *Biological and Chemical Interactions in*

*the Rhizosphere. Symposium Proceedings*, pp. 115-136. Stockholm, Sweden: Swedish National Scientific Research Council Press.

**Tsutsumi M. 1976.** Conditions for collecting the potato root diffusate and the influence on the natural hatching of potato cyst nematode. *Japanese Journal of Nematology* **6**:10-13.

**Vestberg M. 1992.** The effect of vesicular-arbuscular mycorrhizal inoculation on the growth and root colonisation of ten strawberry cultivars. *Agricultural Science in Finland* **1**:527-534.

**Whitehead A G. 1992.** Emergence of juvenile PCN, *Globodera rostochiensis* and *G. pallida* and the control of *G. pallida*. *Annals of Applied Biology* **120**:471-486.

## **Chapter C**

### **Variation in plant growth and *in vitro* hatching activity towards potato cyst nematodes in the response of different potato cultivars to inoculation with arbuscular mycorrhizal fungus isolates**

By T DELIOPOULOS, P P J HAYDOCK and P W JONES

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All the research described in this chapter was carried out by TD

## Summary

Inoculation of six potato (*Solanum tuberosum* L.) cultivars (Home Guard, British Queen, Bintje, Maris Piper, Pentland Dell and Saturna) with Vaminoc (a commercial mixture of three selected isolates of *Glomus* spp.) and with the individual arbuscular mycorrhizal fungi (AMF) isolates *Glomus intraradices* (BioRize BB-E) and *Glomus mosseae* (isolate BEG 12) (components of Vaminoc) stimulated the early hatch of *Globodera pallida* (but not *Globodera rostochiensis*) in potato root leachate (PRL) from 3-week old potato plants, without causing negative effects on plant growth. The mycorrhiza-induced stimulation of *G. pallida* hatch varied with the AMF isolate and cultivar used. Inoculation of potato roots with the mycorrhizal fungal isolate *Gigaspora rosea* (isolate BEG 9) resulted in lower % AMF colonisation (approximately 15% compared to approximately 50% for the other single-isolate AMF inocula) and did not produce any significant effects on PCN hatch. In PRL from 6-week old plants, there was less evidence of *G. pallida* hatch stimulation by AMF. By this time, the stimulatory effect of AMF was restricted to *G. rostochiensis* and was exhibited only by the Vaminoc-treated plants. From the growth parameters measured in plants 6 weeks after shoot emergence, the one most affected by mycorrhizal inoculation was root dry weight (increased). However, this increase also depended on the AMF isolate and on the cultivar used. Colonisation of wheat roots by Vaminoc was not affected by inoculation with *G. pallida* or *G. rostochiensis* nor had Vaminoc inoculation of wheat any significant effect on the hatch of the two potato cyst nematode species. The results of this study indicated that AMF may offer the possibility of being used as part of an integrated pest management strategy for *G. pallida*, by eliminating the usual delay of *G. pallida*

hatch in the field and consequently, by increasing the effectiveness of granular nematicides.

**Key words:** potato cyst nematode, hatch, potato root leachate, arbuscular mycorrhizal fungi, root length colonisation, plant growth, potato cultivars.

## Introduction

Potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) and *Globodera pallida* (Stone), are the most problematic pests of potatoes, with reported annual losses of 9% of potato production in the UK (Evans & Brodie, 1980). The corresponding losses at market value were nearly £43m based on the mean value of the crop from 1990-1995 (Haydock & Evans, 1998). The continuous cropping of *G. rostochiensis*-resistant cultivars has selected for the presence of *G. pallida* in mixed populations and consequently *G. pallida* is now the predominant species in many potato-growing regions (Minnis *et al.*, 2002). *Globodera pallida* is harder to control than *G. rostochiensis*, as, unlike for *G. rostochiensis*, there are no potato cultivars fully resistant to *G. pallida* (Evans & Haydock, 2000). Furthermore, *G. pallida* is more persistent in the soil in the absence of the host crop during crop rotation (due to lower spontaneous hatch; Whitehead, 1992) and hatches later and over a longer period than *G. rostochiensis* (Haydock & Evans, 1998); by the time the majority of *G. pallida* second stage juveniles (J<sub>2</sub>s) hatch, granular nematicides may have already degraded to non-effective concentrations in the soil (Evans & Haydock, 2000).

Hatching of J<sub>2</sub>s from encysted eggs in both species of PCN is stimulated by exposure of eggs to potato root leachate (PRL) (Perry, 1989), which contains

chemicals termed hatching factors (HFs) (Devine, Byrne, Maher & Jones, 1996; Jones, Tylka & Perry, 1998; Devine & Jones 2000*a,b*). Other hatching chemicals present in PRL include hatching inhibitors (HIs: chemicals which reversibly inhibit HF-induced activity), and hatching factor stimulants (HSs: chemicals which stimulate HF-induced hatch without having any effect in isolation on hatching) (Byrne, Twomey, Maher, Devine & Jones, 1998). Byrne, Maher & Jones (2001) found that *G. pallida* and *G. rostochiensis* responded differently to individual HFs isolated from PRL. Hatching activity of PRL to PCN also varies with the potato cultivar used (Evans, 1983); according to Turner & Stone (1981), such variation is attributed to differences in plant growth rather than to variations in the production of hatching activity *per se*, although Devine & Jones (2001) have since demonstrated genetic segregation of HF production in F<sub>1</sub> *Solanum* inter-specific hybrid populations.

The apparently poor control of *G. pallida* by conventional methods has inevitably led potato growers to seek alternative strategies for managing this pest. Integration of partially effective control methods, such as crop rotation, nematicide application and the use of partially resistant cultivars, offers the best prospect for the control of *G. pallida* (Alphey, Robertson & Lyon, 1988). Accelerating the in-soil hatch of *G. pallida* so that it occurs before the degradation of nematicides could improve the effectiveness of these products towards *G. pallida*. Biological HF vector systems, such as soil microorganisms, including selected isolates of rhizobacteria and arbuscular mycorrhizal fungi (AMF) have been shown to increase the hatching activity of PRL. Carroll (1995) and Cronin *et al.* (1997) have isolated bacteria from the plant rhizosphere which were capable of stimulating PCN hatch by producing HFs. Ryan & Jones (2003) found more HFs and HSs, but fewer HIs, in PRL

collected from aseptically-cultured plants co-cultivated with tuber bacteria than from plants grown without the bacteria. Ryan, Duffy, Cassells & Jones (2000) and Ryan, Deliopoulos, Jones & Haydock (2003) demonstrated that inoculation of the maincrop potato cultivar Golden Wonder with Vaminoc, a commercial mixed-isolate population of three *Glomus* spp., increased early hatch of *G. pallida* (*in vitro* and in-soil; Ryan *et al.*, 2000), tolerance to *G. rostochiensis* and multiplication of *G. rostochiensis* without inducing negative effects on plant growth and development (Ryan *et al.*, 2003).

All previous studies by this research group on the AMF-potato-PCN interaction involved a single potato cultivar, Golden Wonder. For AMF-accelerated *G. pallida* hatch to be considered as part of an integrated management for PCN control in commercial potato crops, the effect would need to be expressed over a wide range of potato cultivars. This study was designed to determine the cultivar x AMF interactions on PCN hatch. A second, smaller, experiment was also conducted with the objective of investigating the PCN hatching response to mycorrhizal inoculation of roots of wheat (PCN non-host) in order to test whether the AMF effects on hatch were host-specific.

## **Materials and Methods**

### **Potato *in vitro* hatch experiment**

#### **Plant material**

Certified disease-free tubers (15-25 mm) of six potato cultivars were used: the first early cv. Home Guard (HG), the second early cvs Bintje (BI) and British Queen (BQ) and the maincrop cvs Maris Piper (MP), Pentland Dell (PD) and Saturna (SA). Potato cvs Maris Piper and Saturna are resistant to *Globodera rostochiensis* but



susceptible to *Globodera pallida*, while the other cultivars are susceptible to both PCN species. Tubers were chitted for 4 wk in cool, dry conditions at 10-15°C until they produced strong, sturdy sprouts 2-3 cm long. They were then planted in trays (1.2 x 0.6 x 0.2 m) of washed quartz sand (Supamix™, Pioneer Supamix Ltd., Warwickshire, UK) at a depth of 5 cm and fed every 2 days from planting with nutrient solution (NPK 10.0:4.4:22.4; Phostrogen Ltd., Corwen, UK), pH 6.0 at a rate of 0.5 g/l water per tray (2 liters per tray). Tubers were maintained for 2 wk in the trays to allow sufficient root development before AMF inoculation.

### AMF isolates

The four AMF inocula used were Vaminoc, a commercial mixture of three selected isolates of *Glomus* species, *Glomus intraradices* (BioRize BB-E), *Glomus mosseae* (isolate BEG 12) and *Gigaspora rosea* (isolate BEG 9) and were supplied by BioRize (Dijon, France). The *G. intraradices* and *G. mosseae* isolates are present in the Vaminoc mixture, unlike *G. rosea*. Except for *G. rosea*, which was supplied as an aqueous spore suspension, all the other AMF isolates were supplied as granular inocula. Each granular AMF inoculum, which had been isolated from the rhizosphere of sorghum (*Sorghum vulgare*) plants, contained a mixture of mycorrhizal spores, hyphae and infected root segments, and the propagule carrier was calibrated (0.5-2.5 mm) calcined clay particles (B Blal, BioRize, personal communication).

### Inoculation with AMF

During transplantation to 11.5 cm pots of washed quartz sand, each tuber (one per pot) was separately inoculated with 1 g of Vaminoc, *G. intraradices* or *G. mosseae* granular inoculum, or with 60 spores of *G. rosea*. For the former three

AMF, the inoculum was poured into the planting hole at a depth of 5 cm in each pot. The sprouted tuber was then placed in the hole with the developing roots in contact with the inoculum. For inoculation with *G. rosea*, the tuber was placed in the planting hole and the requisite volume of spore suspension was pipetted onto the developing roots. For control plants, no AMF inoculum was added. The pots were incubated under glasshouse conditions (minimum temperature 16°C, 16 h photoperiod, daylight supplemented by high-pressure 400 W sodium vapour lamps). There were six replicate pots of each of the 30 samples (six cultivars x five treatments) arranged in a replicated randomised block design. The plants were each fed every 2 days with 320 ml of the nutrient solution described above.

### **Determination of AMF root colonisation**

For assessing AMF root colonisation, root systems were removed from each plant 6 wk after shoot emergence and washed gently with tap water to remove sand. Following this, the roots were randomly dispersed in 14 cm diameter round Petri dishes in distilled water, chopped into 2-4 cm long segments and a 2 g root sample per root was collected at random. The root sample from each replicate plant was then placed into a test tube containing 10% (w/v) KOH solution and cleared in an autoclave cycle of 20 minutes at 121°C. After the KOH was drained off, the root pieces were captured on a 250 µm sieve and rinsed thoroughly with tap water. This was followed by acidification of the root samples in their test tubes with 1% (v/v) HCl for 3 minutes. After the HCl was poured off, the root pieces were stained with 0.05% (w/v) Trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and distilled water) (Brundrett, Melville & Peterson, 1994) and left overnight at 4°C (Kormanik & McGraw, 1982). The root samples were then washed with distilled water and

immersed in 50% (v/v) glycerol for 5 days to allow excess stain to leach from the roots (Brundrett *et al.*, 1994). The percentage AMF root colonisation was determined using a modification of the grid line intersect method of Phillips & Hayman (1970), as employed by Giovannetti & Mosse (1980). From each stained mycorrhizal plant root, ten random 1 cm long segments were excised, mounted on glass slides and observed under a light microscope. A total number of 100 segments, each 0.1 cm long, were observed per plant and, depending on the presence or absence of mycorrhizal structures (hyphae, arbuscules and/or vesicles), each segment was recorded as being either “infected” or “uninfected”. Percentage root colonisation by AMF was calculated as the percentage of root segments which were infected (Nicolson, 1960).

### **Potato root leachate production**

Potato root leachate was collected from each replicate pot 3 and 6 wk after shoot emergence by watering each plant with 320 ml of 0.5 g/l nutrient solution and collecting the first 160 ml (the volume required to saturate the sand in the pot) leached into a beaker placed under the pot. The collected leachates were filtered through 3 MM Whatman filter paper to remove sand and other particulate matter and stored at 4°C until needed. For the *in vitro* hatching bioassays, each PRL sample was diluted five-fold with distilled water.

### **Assessment of AMF effects on plant growth**

Six weeks after shoot emergence, plants were harvested and morphometric studies were conducted to determine the physical and physiological responses of potato plants to inoculation with AMF. The Minolta chlorophyll meter SPAD-502

(Spectrum Technologies, Illinois, USA) was used to determine chlorophyll content (measured in arbitrary SPAD units); six measurements were taken on the first fully expanded leaf of each plant. Plant height was also measured, while total leaf area was assessed using the 2-D image analysis system DIAS 1.07 (Delta-T Devices, Cambridge, UK). For each plant, total leaf, shoot and root dry weight were determined after oven-drying of the respective tissues at 60°C for 48 h; allowance was made for the root sample taken for estimation of AMF root colonisation when calculating root dry weight.

### **Nematodes**

Single-generation 2-year-old cysts of *G. pallida* (pathotype Pa2/3) and *G. rostochiensis* (Ro1) were used (C Fleming, DARDNI, Northern Ireland). The cysts were pre-soaked on filter paper discs saturated with distilled water at 22°C for 7 days prior to use.

### **Hatching assay procedure**

The assay procedure followed in this study was based on the microplate bioassay method of Twomey, Raftery, Devine & Jones (1995). The cyst incubation unit was a microtitre plate (disposable sterile ELISA plate; Corning, New York, USA) containing 96 flat-bottomed wells. Cysts were immersed in 200 µl aliquots of the test solution or water controls in individual wells. Hatching activity was calculated as the percentage of viable J<sub>2</sub>s which hatched from three replicate batches each of five cysts after incubation in the test sample at 22°C for 21 days in a humid chamber. To enable calculation of percentage hatch, the number of hatched J<sub>2</sub>s and the number of viable eggs remaining in the cysts at the end of the experiment were

counted. For the viability tests, cysts were immersed in 0.1% (w/v) aqueous Meldola's blue solution (Shepherd, 1962) for 7 days. The cysts were then washed and soaked in water overnight to remove excess stain. Following this, they were placed in 200 µl water in 1.5 ml Eppendorf tubes and crushed gently with an Eppendorf homogeniser to free the eggs. Three 20 µl aliquots were taken after the egg suspension had been thoroughly mixed using a vortex mixer and the number of unstained and partially stained unhatched eggs were recorded as being viable.

### **Wheat in-soil hatch experiment**

Seeds of the wheat (*Triticum aestivum*) cultivar Guardian were pre-soaked on distilled water-saturated discs of filter paper and left overnight at 4°C. They were then sown in trays (0.63 x 0.63 x 0.2 m) containing washed quartz sand and fertilised at planting and every 2 days thereafter with 0.25 g/l of the commercial nutrient solution described earlier. Seedlings emerged after 2 wk of incubation in the trays under standard glasshouse conditions. During transplantation to 11.5 cm pots of the same support medium (i.e. washed quartz sand), seedlings were separately inoculated with 1 g Vaminoc inoculum and 60 cysts of *G. pallida* or *G. rostochiensis* or with 1 g Vaminoc only or with 60 cysts of *G. pallida* or *G. rostochiensis* only. Pots containing plants only or containing cysts of *G. pallida* only or of *G. rostochiensis* only were also set up making a total of eight treatments for the entire experiment. The methodology followed for mycorrhizal and PCN inoculation of the wheat seedlings was as described by Ryan *et al.* (2003). The experimental units (potted plants) were incubated outside in the experimental garden of the Department of Plant Science (The National University of Ireland, Cork) in a replicated randomised design. Each of the eight treatments was replicated five times for each of the two sampling dates

(4 and 12 wk after planting) making a total of 80 pots for the entire experiment. The pots were covered with white oilcloth to reflect sunlight and minimise temperature elevation. During the 12 wk growing period, plants were fertilised every second day starting from planting with nutrient solution (Phostrogen Ltd, Corwen, UK) at a rate of 0.5 g/l of water. At each sampling date, percentage hatch of viable eggs and percentage root length colonisation by Vaminoc were determined using the methodology described earlier.

### **Data analysis**

Statistical analysis on the data was conducted by parametric analysis of variance (ANOVA) using the Genstat Release 4.2 programme for Windows (Lane & Payne, 1996). Where required, multiple comparison tests between more than two treatment means were carried out using the Tukey test. Total leaf area data (Table 2) were normalised by square root transformation prior to parametric analysis.

## **Results and Discussion**

### **Root colonisation by AMF**

Microscopic observations of the root systems sampled 6 wk after shoot emergence showed that all AMF isolates colonised successfully the roots of all potato cultivars tested. The mean values and the interaction ANOVA results are summarised in Table 1.

There was a significant AMF main effect on percentage root colonisation ( $P < 0.001$ ). Inoculation of roots with the aqueous spore suspension of *Gigaspora rosea* resulted in significantly lower ( $P < 0.001$ ) colonisation (grand mean 15.19%),

**Table 1. Percentage arbuscular mycorrhizal fungi (AMF) root colonisation in the potato cultivars Home Guard (HG), British Queen (BQ), Bintje (BI), Maris Piper (MP), Pentland Dell (PD) and Saturna (SA) 6 weeks after shoot emergence**

Cultivar	AMF inoculation status			
	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>	<i>G. rosea</i>
HG	68.67 j	62.17 ij	58.17 hi	15.83 a
BQ	54.50 gh	49.33 cdefg	51.67 efgh	16.50 a
BI	53.00 fgh	50.50 efg	47.50 bcdefg	16.00 a
MP	52.67 fgh	49.17 defg	51.67 efgh	14.33 a
PD	41.50 b	45.00 bcde	43.67 bcd	14.17 a
SA	52.00 efgh	42.33 bc	46.33 bcdef	14.33 a
Source of variation	df	<i>F</i> -ratio	<i>Probability</i>	
Cultivar	5	13.69	< 0.001	
AMF	3	254.72	< 0.001	
Cultivar x AMF	15	1.93	< 0.05	
Error	120	-	-	

Any two treatment means sharing a common letter were not significantly different at the  $P = 0.05$  level, using the Tukey test

compared to the other three mycorrhizal inocula, Vaminoc (53.72%), *G. intraradices* (49.75%) and *G. mosseae* (49.83%). The latter three inocula, available commercially, are obtained from mycorrhizal plants grown in soil pot cultures. Such granular AMF inocula will have a dense, pre-existing network of AMF hyphae resulting from the previous root activity, in comparison with aqueous AMF formulations containing only AMF spores, such as *G. rosea* in this study. Therefore, granular AMF inocula would be expected to contain an increased population of mycorrhizal infective units at inoculation stage and hence to colonise host roots more rapidly and extensively than an inoculum consisting of a spore suspension. The grand mean percentage root colonisation by Vaminoc was significantly higher ( $P < 0.05$ ) than that by *G.*

*intraradices* and *G. mosseae*, suggesting a synergistic interaction between the three isolates present in Vaminoc, although the non-inclusion of the third Vaminoc isolate (*Glomus dussii*) in these trials makes interpretation difficult.

The main effect of cultivar on percentage root colonisation was also highly significant ( $P < 0.001$ ), indicating that the roots of the six potato cultivars responded differently to mycorrhizal inoculation, a result largely expected considering the variations that occur between potato cultivars in the structure of the root system (e.g. extensiveness, geometry and/or epidermis structure). Home Guard had the highest mean percentage colonisation among potato cultivars (grand mean 51.21%; significantly higher than the other cultivars,  $P < 0.001$ ) and Pentland Dell had the lowest (grand mean 36.08%; significantly lower ( $P < 0.05$ ) than all other cultivars apart from cv. Saturna). There was also a significant cultivar x AMF interaction ( $P < 0.05$ ), indicating that the cultivars supported different levels of AMF colonisation. Interestingly, there was a close negative relationship between maturity date of the cultivar and colonisation level; the cultivars are listed in Table 1 in order of maturity date (Home Guard is a first early, for example), suggesting that maturity date could affect root development and hence, AMF colonisation.

The differences in the extent of root colonisation among the three single-isolate AMF inocula used in this study could be attributed to functional differences between AMF species, as emphasised by van der Heijden *et al.* (1998); according to Koch, Kuhn, Jansa & Sanders (2001), such differences are more likely to be the result of genetic variation among AMF species rather than the result of differences in the environment from where the AMF were isolated. Assessment of mycorrhizal colonisation in the roots of wheat plants cv. Guardian showed that colonisation was



not affected by PCN inoculation at either harvest date (i.e. 4 and 12 wk after planting).

### **Morphological and physiological studies**

Several of the characters assessed were not affected by AMF inoculation in any of the cultivars examined. Chlorophyll content and total leaf area of plants 6 wk after shoot emergence were not significantly affected by mycorrhization. Furthermore, the only cultivar in which shoot height was significantly affected (increased) was cv. Home Guard, when Vaminoc and *G. rosea* were the inocula ( $P < 0.05$ ; Table 2a). Mycorrhizal inoculation of roots of cv. Maris Piper with any of the four AMF isolates had a significant detrimental effect with regard to both leaf and shoot dry weight (Table 2d). However, mycorrhizal plants of cv. Maris Piper exhibited increased root dry weights in comparison with non-mycorrhizal plants; this increase was significant ( $P < 0.05$ ; Table 2d) for Vaminoc- and *G. rosea*- treated plants. Apart from cv. Maris Piper, in none of the other cultivars did AMF inoculation of potato roots have any significant effect on the shoot dry weight of plants. There were six other cases where root inoculation with an AMF isolate had a significant positive effect ( $P < 0.05$ ) on plant growth. These were leaf dry weights of cvs Home Guard, British Queen and Pentland Dell treated with *G. mosseae*, Vaminoc and *G. intraradices*, respectively, and root dry weights of cv Home Guard treated with Vaminoc and *G. intraradices*, and of cv. Saturna treated with Vaminoc (Table 2).

**Table 2. Growth response of the potato cultivars (a) Home Guard (HG), (b) British Queen (BQ), (c) Bintje (BI), (d) Maris Piper (MP), (e) Pentland Dell (PD) and (f) Saturna (SA) to inoculation with arbuscular mycorrhizal fungi (AMF) 6 weeks after shoot emergence [Shoot Height (SH): cm, Leaf Dry Weight (LDW): g, Shoot Dry Weight (SDW): g, Root Dry Weight (RDW): g, Chlorophyll Content (CC): SPAD units, Total Leaf Area (TLA): cm<sup>2</sup>]**

Cultivar	AMF inoculation status					P
(a) HG	Control	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>	<i>G. rosea</i>	
SH	11.7a	15.5b	13.7ab	14.3ab	15.5b	*
LDW	0.345a	0.429ab	0.359a	0.499b	0.342a	*
SDW	0.221	0.266	0.256	0.306	0.300	NS
RDW	0.068a	0.088b	0.084b	0.069a	0.080ab	*
(b) BQ	Control	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>	<i>G. rosea</i>	
SH	12.8	12.3	13.3	13.1	12.3	NS
LDW	0.248ab	0.310c	0.292bc	0.219a	0.265abc	*
SDW	0.327	0.345	0.333	0.317	0.307	NS
RDW	0.185	0.173	0.177	0.249	0.186	NS
(c) BI	Control	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>	<i>G. rosea</i>	
SH	19.2	20.1	21.4	20.2	18.9	NS
LDW	0.196	0.180	0.151	0.150	0.204	NS
SDW	0.287	0.267	0.274	0.272	0.200	NS
RDW	0.096	0.098	0.106	0.085	0.110	NS
(d) MP	Control	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>	<i>G. rosea</i>	
SH	26.2	22.9	20.0	21.9	19.5	NS
LDW	0.396c	0.163a	0.234b	0.226b	0.219b	***
SDW	0.344b	0.226a	0.242a	0.190a	0.228a	**
RDW	0.068a	0.098b	0.084ab	0.079a	0.097b	**
(e) PD	Control	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>	<i>G. rosea</i>	
SH	13.5	15.7	13.6	15.3	15.6	NS
LDW	0.257b	0.272b	0.328c	0.235ab	0.198a	***
SDW	0.200	0.228	0.200	0.206	0.196	NS
RDW	0.063	0.067	0.058	0.063	0.071	NS
(f) SA	Control	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>	<i>G. rosea</i>	
SH	10.4	9.0	10.4	9.1	9.4	NS
LDW	0.415b	0.488b	0.492b	0.443b	0.293a	*
SDW	0.323	0.309	0.339	0.271	0.293	NS
RDW	0.156a	0.229b	0.181a	0.157a	0.137a	**

Interaction ANOVA summary: <i>F</i> -ratios (probability in parentheses; error df = 150)			
Character	Cultivar <sup>5</sup> df	AMF <sup>4</sup> df	Cultivar x AMF <sup>20</sup> df
CC	18.68 (< 0.001)	5.56 (< 0.001)	1.11 (> 0.10)
TLA <sup>a</sup>	58.45 (< 0.001)	1.86 (> 0.10)	1.02 (> 0.10)
SH	46.71 (< 0.001)	0.19 (> 0.10)	1.05 (> 0.10)
LDW	60.42 (< 0.001)	4.53 (< 0.01)	5.19 (< 0.001)
SDW	17.66 (< 0.001)	1.59 (> 0.10)	2.28 (< 0.01)
RDW	110.97 (< 0.001)	2.22 (< 0.10)	3.49 (< 0.001)

Treatment means within a row followed by the same letter were not significantly different at the  $P = 0.05$  level, using the Tukey test

<sup>a</sup> Data square-root transformed (to normalise) prior to parametric analysis

NS, \*, \*\*, \*\*\* = Non-significant or significant at the  $P = 0.05$ ,  $P = 0.01$  or  $P = 0.001$  levels, respectively

Analysis of the two main effects (cultivar and AMF) and the interaction revealed that, averaged over the six cultivars, AMF inoculation had significant effects on chlorophyll content and leaf dry weight, while the effect on root dry weight was near-significant ( $P = 0.07$ ). For each of the six parameters, however, the cultivar main effect was considerably larger than the AMF main effect, indicating that the influence of the mycorrhizal fungi was relatively small. All three biomass parameters (leaf, shoot and root dry weight) exhibited significant cultivar x AMF interactions (Table 2).

The results on the biomass parameters indicated that mycorrhizal inoculation of potato plants had little or almost no effect on the growth of four cultivars (cvs British Queen, Bintje, Pentland Dell and Saturna), but had a stimulatory effect on the growth of cv. Home Guard and an inhibitory effect on the growth of cv. Maris Piper. The character mostly affected by AMF inoculation was root dry weight.

The stimulatory effects of AMF on root growth might have been the result of an increased branching of the mycorrhizal roots, resulting in greater proportion of

higher order roots in mycorrhizal than in non-mycorrhizal plants. However, this hypothesis could not be verified, because, as in most AMF studies, examination of root anatomical changes was not conducted. Atkinson, Berta & Hooker (1994) reported that mycorrhizal colonisation induces remarkable changes in root system morphology by increasing the meristematic and nuclear activities of the root cells. In the current study, in most cases, the increase in root growth of mycorrhizal plants (relative to non-mycorrhizal controls) was not reflected in a similar increase in the above-ground growth parameters. A possible explanation for that would be that the mycorrhizal fungi may have increased the absorption of nutrients (phosphorus in particular) from the surrounding soil but they may have used these nutrients more for their own growth and metabolism within the root cells instead of transporting them to the plant. It could also be that mycorrhizal activity may have induced an increase in the production of plant growth regulators such as cytokinins and gibberellins in the root system. The lack of a stimulatory effect of AMF on shoots and leaves could be simply due to the early stage at which plants were harvested (i.e. 6 wk after shoot emergence), by which time the beneficial effects of the AMF-plant symbiosis had not yet been apparent on the above-ground sections of the plant or due to a minimal effect by the mycorrhizal fungus on the production of plant hormones in leaves and shoots. The hypothesis that AMF might have played a key role in the production of phytohormones has been well documented, although little has been published, relative to other plants, on the potato plant-AMF symbiosis. Shaul-Keinan *et al.* (2002) reported significant changes in the concentration of auxins, cytokinins and gibberellins in tobacco (*Nicotiana tabacum*) plants at the early stages of colonisation by *G. intraradices* but they observed that these hormonal changes during the fungal-host symbiosis occurred well before the fungal benefits manifested. Allen, Moore &

Christensen (1980) demonstrated altered cytokinin levels in *Bouteloua gracilis* plants resulting from mycorrhizal infection; the increase in total cytokinin activity in mycorrhizal over control plants, was greater in the roots (111%) than in the leaves (57%).

The fact that different potato cultivars responded differently to mycorrhizal inoculation although grown under similar conditions (growth substrate, environment) and the observation that each AMF isolate produced a different plant response, demonstrated an AMF-host (i.e. potato cultivar)-specific effect. For example, inoculation of potato roots with Vaminoc stimulated the root growth in three cultivars (cvs Home Guard, Maris Piper and Saturna), but had no effect on the root growth of the other cultivars. The reason for this AMF-host specificity is not fully understood. It may be possible that variations in the structure of the root system among potato cultivars could influence the capacity of AMF to absorb nutrients and transfer them to the plant; Curl & Truelove (1986) emphasised that the capacity of mycorrhizal plants to influence nutrient availability in soils and hence, plant growth, is highly dependent on the activity and the extensiveness of the root systems. Johnson & Pfleger (1992) reported that an efficient mycorrhizal root coloniser may not be an effective symbiont for the plant. On the other hand, the degree of growth enhancement in the same plant depends largely on the AMF species present (Davis & Menge, 1981).

The most positive AMF-cultivar association observed in this study in promoting plant growth was the Vaminoc-cv. Home Guard association. There is evidence in the literature that certain mycorrhizal fungus-host associations are more efficient than others in enhancing plant growth. Guillemain, Gianinazzi & Trouvelot (1992), investigating the interaction between three micropropagated pineapple

cultivars and five AMF isolates, found that certain AMF-cultivar associations promoted plant growth while others did not. Interestingly, cv. Home Guard, a first early maturing cultivar (the earliest of all the cultivars investigated), also exhibited the highest levels of root colonisation by the three granular AMF inocula among all potato cultivars, suggesting that beneficial AMF-cultivar interactions could be identified early by assessing percentage root colonisation. Furthermore, potato plant benefits provided by mycorrhizae may be associated with the maturity level of the cultivar used. When the mean values of total dry biomass produced were compared between AMF-untreated and AMF-treated plants (average of all four AMF) for each cultivar individually, it was revealed that mycorrhization had a positive effect with respect to total dry weight only in cv. Home Guard ( $t = 3.134$ ,  $df = 28$ ,  $P < 0.01$ ), the only first early cultivar used; in second earlies and maincrop cultivars mycorrhizal inoculation had either a negative (cv. Maris Piper,  $t = 7.301$ ,  $df = 28$ ,  $P < 0.001$ ) or a neutral effect. This finding suggests that the beneficial effects of the mycorrhizae-plant symbiosis on plant growth could be greater in early maturing than late maturing cultivars.

### Percentage hatch

Only under non-mycorrhizal plants was the difference in mean percentage hatch (estimated from all cultivars and PRL collection dates) between *G. rostochiensis* and *G. pallida* significant ( $P < 0.001$ ; *G. pallida*: 68.2%, *G. rostochiensis*: 82.5%). The grand mean percentage hatch of *G. rostochiensis* (85.7%), calculated from all cultivars, AMF-treated and AMF-untreated plants and PRL collection dates (Fig. 1), was still significantly greater ( $P < 0.001$ ) than that of *G. pallida* (77.4%), but this time the difference in the mean hatch values between the

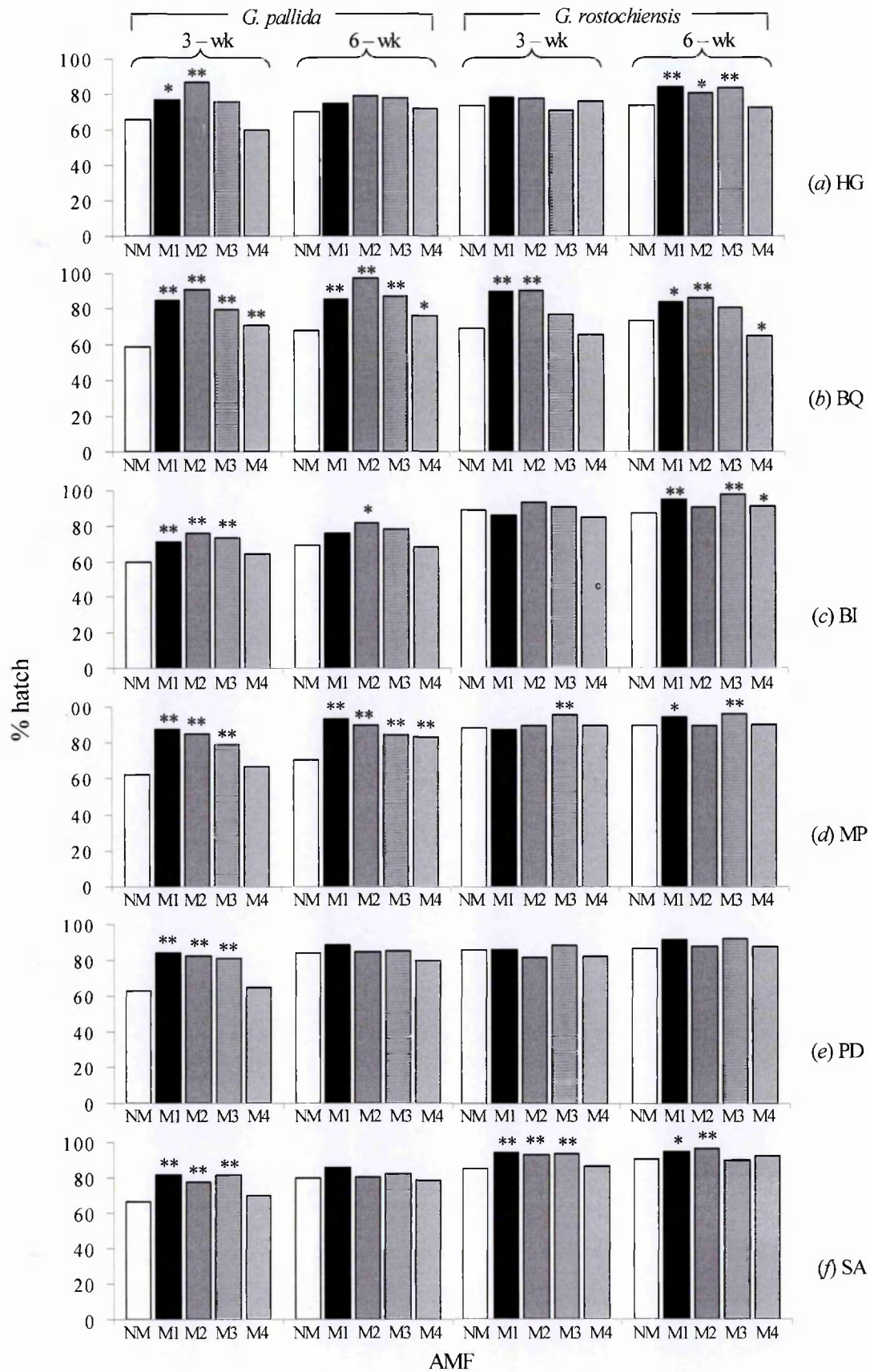


Fig. 1

two PCN species was smaller, as compared to the control, indicating that AMF inoculation of the potato root at the time of planting reduced the difference between *G. pallida* and *G. rostochiensis* hatch.

The ANOVA (Table 3) showed that there was a significant AMF main effect on percentage hatch ( $P < 0.001$ ), with AMF-treated plants stimulating greater hatch than AMF-untreated plants. The AMF effect depended on date (AMF x date interaction,  $P < 0.01$ ) and PCN species (AMF x PCN,  $P < 0.001$ ). The significant cultivar x PCN x AMF interaction ( $P < 0.001$ ) indicated that the different hatch response of the two PCN species to AMF inoculation was cultivar-dependent.

The percentage hatch of viable eggs of *G. pallida* exposed to 3 wk-PRL (i.e. PRL collected 3 wk after shoot emergence) was greater in PRL from plants treated with the three granular mycorrhizal inocula, than in PRL from non-mycorrhizal plants. By this time, the increase in the hatch of *G. pallida* was significant ( $P < 0.05$  or  $P < 0.01$ , depending on the AMF-cultivar association; Fig. 1) in 17 out of the 18 possible granular AMF-cultivar associations and marked (but not significant) in the remaining combination, *G. mosseae*-cv. Home Guard association (Fig. 1). Unlike the situation with the granular AMF inocula, after 3 wk, inoculation of potato roots with the *G. rosea* aqueous spore suspension caused a significant effect on the hatch of *G. pallida* only in cv. British Queen (hatch significantly greater than control,  $P < 0.01$ ; Fig. 1). The lower stimulation of *G. pallida* hatch in PRL from 3-wk *G. rosea*-treated plants, compared to that in PRL from plants treated with the granular AMF inocula, was associated with significantly reduced root colonisation by *G. rosea* and was possibly the result of this effect.

After 6 weeks, there was less evidence of *G. pallida* hatch stimulation by AMF. By this time, only in cvs British Queen and Maris Piper did mycorrhizal



inoculation of the root system by all four AMF inocula induce significantly increased *G. pallida* hatch, relative to control plants ( $P < 0.05$ ; Fig. 1). Interestingly, after 6 wk there was a significant increase in *G. pallida* hatch (relative to 3 wk) only in leachates collected from non-mycorrhizal roots, so that by this time, percentage hatch was similar in PRL from AMF-treated and AMF-untreated plants of all cultivars except cvs British Queen and Maris Piper (Fig. 1).

The fact that from 3 to 6 wk the hatch of *G. pallida* was increased only in leachates from non-mycorrhizal roots demonstrated that there was a delay in the hatch of *G. pallida*. The increased *G. pallida* hatch in 3-wk PRL collected from plants treated with the three granular AMF preparations, compared to that from non-mycorrhizal plants, demonstrated that mycorrhizal inoculation (granular but not aqueous) of the potato root system accelerated early *G. pallida* hatch (0-3 wk), but did not result in increased total hatch after 6 wk.

Mycorrhization produced a much smaller effect on the hatch of *Globodera rostochiensis*, compared to that on *G. pallida*, after 3 wk. By this time, in only six cases (Vaminoc- and *G. intraradices*-treated plants of cvs British Queen and Saturna, *G. mosseae*-treated plants of cvs Maris Piper and Saturna) was the hatch of *G. rostochiensis* in response to leachate from mycorrhizal roots significantly greater ( $P < 0.01$ ) than that in leachate from non-mycorrhizal roots (Fig. 1). At 6 weeks, though, there was evidence for Vaminoc-induced stimulation of *G. rostochiensis* hatch in PRL from all cultivars and a few cases of hatch stimulation by the single-AMF isolates *G. intraradices* and *G. mosseae* (Fig. 1). Unlike *G. pallida*, there was no increase from 3 to 6 wk in the *in vitro* hatch of *G. rostochiensis* in root leachate

**Fig. 1.** *In vitro* hatch of *G. pallida* and *G. rostochiensis* J<sub>2</sub>s after 21 days of incubation in potato root leachates (PRL) collected 3 and 6 weeks after shoot emergence from mycorrhizal (M1: Vaminoc, M2: *Glomus intraradices*, M3: *Glomus mosseae*, M4: *Gigaspora rosea*) and non-mycorrhizal (NM) plants of the potato cultivars (a) Home Guard (HG), (b) British Queen (BQ), (c) Bintje (BI), (d) Maris Piper (MP), (e) Pentland Dell (PD) and (f) Saturna (SA). \*, \*\* = significantly different from NM at the  $P = 0.05$  or  $P = 0.01$  levels, respectively, using the Tukey test.

**Table 3. Interaction analysis of variance of percentage hatch for data in Fig. 1**

Source of variation	df	<i>F</i> -ratio	<i>Probability</i>
Cultivar	5	53.33	< 0.001
PCN	1	379.60	< 0.001
Date	1	102.33	< 0.001
AMF	4	116.39	< 0.001
Cultivar x PCN	5	48.78	< 0.001
Cultivar x Date	5	2.19	< 0.10
PCN x Date	1	17.91	< 0.001
Cultivar x AMF	20	6.74	< 0.001
PCN x AMF	4	19.00	< 0.001
Date x AMF	4	4.34	< 0.01
Cultivar x PCN x Date	5	2.09	< 0.10
Cultivar x PCN x AMF	20	3.73	< 0.001
Cultivar x Date x AMF	20	1.57	< 0.10
PCN x Date x AMF	4	5.46	< 0.001
Cultivar x PCN x Date x AMF	20	1.89	< 0.05
Error	600		

collected from AMF-untreated plants of all six cultivars, indicating that the hatch of *G. rostochiensis* (in absence of AMF) was more-or-less completed by wk 3, in contrast with *G. pallida*. The delayed hatch of *G. pallida* compared to *G. rostochiensis* observed in this study has also been reported previously from other *in vitro* studies as well as from in-soil studies (Evans, 1983; Robinson, Atkinson & Perry, 1987; Whitehead, 1992; Ryan *et al.*, 2000; Byrne *et al.*, 2001). Jones *et al.* (1998) associated the delayed hatch of *G. pallida* with the late production of *G. pallida*-preferred HFs in PRL.

Rarely did a single-isolate AMF preparation cause increased hatch when Vaminoc did not; the only two exceptions were the hatch of *G. pallida* in PRL collected 6 wk after shoot emergence from *G. intraradices*-treated plants of cv. Bintje and the *G. rostochiensis* hatch in leachate collected from 3-wk *G. mosseae*-treated plants of cv. Maris Piper.

Differences in the hatching activity of PRL towards the two PCN species were apparent among the six cultivars. British Queen induced significantly greater ( $P < 0.05$ ) hatch of *G. pallida* than of *G. rostochiensis*, whereas the other five cultivars induced significantly greater hatch in *G. rostochiensis* than in *G. pallida* (cv. Home Guard:  $P < 0.01$ , cvs Bintje, Maris Piper, Pentland Dell, Saturna:  $P < 0.001$ ), suggesting increased production of *G. pallida*-preferred HFs in the former and of *G. rostochiensis*-preferred HFs in the latter. Recent studies by this research group (Byrne *et al.*, 2001; Devine & Jones, 2001; Ryan & Jones, 2004) have demonstrated that, at least, some of the multiple HFs produced in PCN-host root leachate showed preferences to one PCN species than another (species-specific or species-selective HFs). There was also evidence to support the findings of Evans (1983) in that PCN hatch in PRL varied with the potato cultivar used. In particular, the hatch of *G.*

*pallida* was significantly higher ( $P < 0.001$ ) in PRL collected from cvs British Queen, Maris Piper, Pentland Dell and Saturna than in PRL from cvs Bintje and Home Guard, while leachate collected from cvs Bintje, Maris Piper and Saturna hatched significantly higher ( $P < 0.01$ ) numbers of *G. rostochiensis* than did the PRL from the other cultivars (i.e. cvs British Queen, Home Guard and Pentland Dell). This effect may have been the result of quantitative and/or qualitative differences in HF production among the six potato cultivars.

The significant increase in the early hatch of *G. pallida* by (granular) AMF inoculation of the root system was not associated (in the majority of the cases, that is in 14 out of 18 cases) with a similar increase in root growth. The most likely scenario for the mycorrhiza-induced *G. pallida* hatch stimulation involves an altered production of hatch-positive chemicals present in the host root leachate as a result of the mycorrhizal activity in the potato rhizosphere. The production of novel mycorrhizal HFs by the granular AMF inocula was far less likely to be the reason for the AMF-induced stimulation of *G. pallida* hatch in 3-wk PRL, because when Vaminoc was inoculated onto the roots of the PCN non-host wheat, growing in the presence of *G. pallida* or *G. rostochiensis* cysts, no increase in the hatch of either PCN species was observed at either harvest date (i.e. after 4 or 12 wk), suggesting that AMF can increase PCN hatch (*G. pallida* in particular) only in plants capable of producing HFs. More recent research (Ryan & Jones, 2004) has shown that the *G. pallida* hatch stimulation following inoculation of the potato root system with Vaminoc was the result of the increased production of mycorrhiza-specific HFs (i.e. absent in the minus-AMF profile) and mycorrhizal-selective HFs (i.e. present in both profiles, but inducing significantly greater hatching activity towards *G. pallida* in the plus-AMF profile) and to the production of an increased amount of HSs in PRL from

AMF-treated plants, compared to AMF-untreated plants. The origin of these mycorrhiza-specific HFs has not yet been identified, but these recent studies have provided increasing evidence that these HFs are more likely to be the result of the plant x AMF symbiosis rather than the result of independent mycorrhizal activity. Therefore, the term 'novel mycorrhizal HFs' should be used with caution.

This study demonstrated that mixed-AMF (Vaminoc) or certain single-isolate AMF preparations (*G. intraradices* and *G. mosseae*) were capable of colonising extensively the potato root system of a wide range of potato cultivars without causing negative effects on plant development. In particular, the effects of AMF on potato plant growth were largely cultivar-independent and, in the majority of the cases, non-mycorrhizal and mycorrhizal plants affected plant growth similarly. The successful colonisation of the root system stimulated the early hatch of *G. pallida* *in vitro*, eliminating in this way the usual delay in *G. pallida* (relative to *G. rostochiensis*) hatch. In conclusion, all AMF increased PCN hatch under certain circumstances (*G. pallida* after 3 wk, *G. rostochiensis* after 6 wk; *Gigaspora rosea* had the least effect).

Although the current results were promising, further research would be necessary before deciding to use AMF as part of an integrated *G. pallida* management strategy plan. The next step in this research would be to analyse the hatching activity of PRL towards PCN from plants colonised with selected AMF inocula (i.e. Vaminoc, *G. intraradices* and *G. mosseae*) in order to investigate whether the AMF-induced hatch of *G. pallida*, as demonstrated in this paper, is associated with increased production of HFs from mycorrhizal plants. The effects of the individual AMF isolates *G. intraradices* and *G. mosseae* on hatching chemical production will be compared with that of Vaminoc, in order to obtain information on the interaction between these isolates in the Vaminoc mixture. Pot and field

experiments in PCN-infested soil would then need to be conducted to determine the effects of applying these selected potato root colonisers in the presence of a nematicide at planting on the control of *G. pallida*. The fact that the stimulatory effect of AMF on early *G. pallida* hatch was expressed over all potato cultivars was a positive result suggesting that, in future in-soil studies of this nature, selection of the potato cultivar should not be a problem.

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### References

- Allen M F, Moore T S Jr, Christensen M. 1980.** Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhizae: I. Cytokinin increases in the host plant. *Canadian Journal of Botany* **58**:371-374.
- Alphey T J W, Robertson W M, Lyon G D. 1988.** Rishitin, a natural plant product with nematocidal activity. *Revue de Nématologie* **11**:399-404.
- Atkinson D, Berta G, Hooker J E. 1994.** Impact of mycorrhizal colonisation on root architecture, root longevity and the formation of growth regulators. In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*, pp. 89-99. Eds S Gianinazzi and H Schüepp. Basel, Switzerland: Birkhäuser.

- Brundrett M C, Melville L, Peterson L. 1994.** *Practical Methods in Mycorrhizal Research*. Waterloo, Canada: Mycologue Publications. 161 pp.
- Byrne J, Maher N, Jones P W. 2001.** Comparative responses of *Globodera rostochiensis* and *G. pallida* to hatching chemicals. *Journal of Nematology* **33**:195-202.
- Byrne J, Twomey U, Maher N, Devine K J, Jones P W. 1998.** Detection of hatching inhibitors and hatching factor stimulants for golden potato cyst nematode, *Globodera rostochiensis*, in potato root leachate. *Annals of Applied Biology* **132**:463-472.
- Carroll M. 1995.** *Characterisation of rhizosphere bacterial isolates which affect the hatch of potato cyst nematodes*. M.Sc. Thesis, The National University of Ireland, Cork, Ireland. 70 pp.
- Cronin D, Moenne-Loccoz Y, Fenton A, Dunne C C, Dowling D N, O’Gara F. 1997.** Role of 2,4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. *Applied and Environmental Microbiology* **63**:1357-1361.
- Curl E A, Truelove B. 1986.** *The Rhizosphere*. Berlin, Germany: Springer-Verlag. 288 pp.
- Davis R M, Menge J A. 1981.** *Phytophthora parasitica* inoculation and intensity of vesicular-arbuscular mycorrhizae in citrus. *New Phytologist* **87**:705-715.
- Devine K J, Byrne J, Maher N, Jones P W. 1996.** Resolution of natural hatching factors for the golden potato cyst nematode, *Globodera rostochiensis*. *Annals of Applied Biology* **129**:323-334.

- Devine K J, Jones P W. 2000a.** Purification and partial characterisation of hatching factors for the potato cyst nematode *Globodera rostochiensis* from potato root leachate. *Nematology* **2**:231-236.
- Devine K J, Jones P W. 2000b.** Response of *Globodera rostochiensis* to exogenously applied hatching factors in soil. *Annals of Applied Biology* **137**:21-29.
- Devine K J, Jones P W. 2001.** Potato cyst nematode hatching activity and hatching factors in inter-specific *Solanum* hybrids. *Nematology* **3**:141-149.
- Evans K. 1983.** Hatching of potato cyst nematodes in root diffusates collected from twenty-five potato cultivars. *Crop Protection* **2**:97-103.
- Evans K, Brodie B B. 1980.** The origin and distribution of the golden nematode and its potential in the USA. *American Potato Journal* **57**:70-89.
- Evans K, Haydock P P J. 2000.** Potato cyst nematode management - present and future. *Aspects of Applied Biology* **59**:91-97.
- Giovannetti M, Mosse B. 1980.** An evaluation of techniques for measuring vesicular-arbuscular infection in roots. *New Phytologist* **84**:489-500.
- Guillemin J P, Gianinazzi S, Trouvelot A. 1992.** Screening of arbuscular endomycorrhizal fungi for establishment of micropropagated pineapple plants. *Agronomie* **12**:831-836.
- Haydock P P J, Evans K. 1998.** Management of potato cyst nematodes in the UK: an integrated approach? *Outlook in Agriculture* **27**:253-260.
- van der Heijden M G A, Klironomos J N, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders I R. 1998.** Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**:69- 72.



- Johnson N C, Pflieger F L. 1992.** Vesicular-arbuscular mycorrhizae and cultural stresses. In *Mycorrhizae in Sustainable Agriculture*, pp. 71-99. Eds G J Bethlenfalvay and R G Linderman. ASA special publication No. 54. Madison, Wisconsin, USA: American Society of Agronomy, Inc.
- Jones P W, Tylka G L, Perry R N. 1998.** Hatching. In *The Physiology and Biochemistry of Free-living and Plant Parasitic Nematodes*, pp. 181-212. Eds R N Perry and D J Wright. Wallingford, UK: CAB International.
- Koch A, Kuhn G, Jansa J, Sanders I R. 2001. A genetic basis for phenotypic variation in a *G. intraradices* population. In *Knowledge on Population Biology of AMF as a tool for Mycorrhizal Technology*, Book of Abstracts. Eds J Rydlova *et al.* Cost 838 Action. Pruhonice, Czech Republic. 26-29 September 2001.
- Kormanik P P, McGraw A C. 1982.** Quantification of vesicular-arbuscular mycorrhizae in plant roots. In *Methods and Principles of Mycorrhizal Research*, pp. 37-45. Ed. N C Schenck. St. Paul, Minnesota, USA: American Phytopathological Society Press.
- Lane P W, Payne R W. 1996.** *Genstat for Windows: an Introductory Course*. Oxford: Numerical Algorithms Group Limited. 154 pp.
- Minnis S T, Haydock P P J, Ibrahim S K, Grove I G, Evans K, Russell M A. 2002.** Potato cyst nematodes in England and Wales - Occurrence and distribution. *Annals of Applied Biology* **140**:187-195.
- Nicolson T H. 1960.** Mycorrhiza in Gramineae II. Development in habitats particularly sand dunes. *Transactions of the British Mycological Society* **43**:132-214.
- Perry R N. 1989.** Dormancy and hatching of nematode eggs. *Parasitology Today* **5**:337-383.

- Phillips J M, Hayman D A. 1970.** Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**:158-161.
- Robinson M P, Atkinson H J, Perry R N. 1987.** The influence of soil moisture and storage time on mobility, infectivity and lipid utilization of the second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Revue de Nématologie* **10**:343-348.
- Ryan N A, Deliopoulos T, Jones P W, Haydock P P J. 2003.** Effects of a mixed-isolate mycorrhizal inoculum on the potato - potato cyst nematode interaction. *Annals of Applied Biology* **143**:111-119.
- Ryan N A, Duffy E M, Cassells A C, Jones P W. 2000.** The effect of mycorrhizal fungi on the hatch of potato cyst nematodes. *Applied Soil Ecology* **15**:233-240.
- Ryan N A, Jones P W. 2003.** Effect of tuber-borne micro-organisms on hatching activity of potato root leachate towards potato cyst nematodes. *Nematology* **5**:55-63.
- Ryan N A, Jones P W. 2004.** The effect of mycorrhization of potato roots on the hatching chemicals active towards the two potato cyst nematode species, *Globodera pallida* and *Globodera rostochiensis*. *Nematology* (in press).
- Shaul-Keinan O, Gadkar V, Ginzberg I, Grunzweig J M, Chet I, Elad Y, Wininger S, Belausov E, Eshed Y, Arzmon N, Ben-Tal Y, Kapulnik Y. 2002.** Hormone concentrations in tobacco roots change during arbuscular mycorrhizal colonization with *Glomus intraradices*. *New Phytologist* **154**:501-507.
- Shepherd A M. 1962.** New Blue R. A stain that differentiates between living and dead nematodes. *Nematologica* **8**:201-208.

- Turner S J, Stone A R. 1981.** Hatching of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) in root exudates of *Solanum vernei* hybrids. *Nematologica* **27**:315-317.
- Twomey U, Raftery T, Devine K J, Jones P W. 1995.** An improved procedure for assaying hatching activity towards *Globodera rostochiensis*. *Nematologica* **41**:258-268.
- Whitehead A G. 1992.** Emergence of juvenile potato cyst nematodes *Globodera rostochiensis* and *G. pallida* and the control of *G. pallida*. *Annals of Applied Biology* **120**:471-486.

## **Chapter D**

### **Studies on the effect of mycorrhization of potato roots on the hatching activity of potato root leachate towards the potato cyst nematodes, *Globodera pallida* and *G. rostochiensis***

By T DELIOPOULOS, K DEVINE, P HAYDOCK and P W JONES

This chapter was submitted to Nematology.

The contribution of KD was in the demonstration of the methodology for G-10 and elemental analysis

## Summary

Successful mycorrhization of potato plants cv. Golden Wonder was achieved with three commercial preparations of arbuscular mycorrhizal fungi (AMF): Vaminoc (mixed-isolate inoculum) and two of its components, *Glomus intraradices* and *G. mosseae*. Mycorrhization increased significantly the root dry weight of plants. Assays of potato root leachates (PRL) for hatching activity towards the two potato cyst nematode (PCN) species, *Globodera pallida* and *G. rostochiensis*, indicated that inoculation with Vaminoc and *G. mosseae* stimulated the hatch of *G. pallida* in the first 3 weeks after shoot emergence. The overall hatch response of *G. rostochiensis* to PRL was greater than *G. pallida* (hatch almost maximum for the former at week 3). When the PRL from week 3 were fractionated by Sephadex G-10 chromatography and standardised by carbon content, multiple AMF effects on hatching factor (HF) production were uncovered. Root leachates from Vaminoc-inoculated plants contained markedly more *G. pallida*-active HFs than all other treatments; in contrast, PRL from the three AMF treatments exhibited little variation in the quantity of *G. rostochiensis*-active HFs produced. Several HFs were PCN species-specific or -selective, with those resolved from the *G. intraradices* and *G. mosseae* PRL profiles exhibiting an apparent preference for *G. rostochiensis* than *G. pallida*.

**Key words:** Golden Wonder, Sephadex G-10 column, arbuscular mycorrhizal fungi, root length colonisation, plant growth

## Introduction

Potato cyst nematodes (PCN), *Globodera pallida* (Stone) and *Globodera rostochiensis* (Woll.), are the most damaging pests of the potato crop in Europe; other commercially grown hosts of PCN include tomato and aubergine. Of the two PCN species, *G. pallida* is the dominant species in major EU potato production areas, primarily because of the widespread cultivation of *G. rostochiensis*-resistant potato cultivars. For example, in England and Wales, Minnis *et al.* (2002) showed that, of the PCN-infested sites sampled, 67% were pure *G. pallida*, 8% were pure *G. rostochiensis*, and 25% contained a mixture of the two PCN species.

Hatch of J<sub>2</sub>s occurs in response to multiple HFs, which are chemicals leaching from roots of host plants (Devine *et al.*, 1996; Jones *et al.*, 1998). Furthermore, Devine *et al.* (1996) reported that root leachate collected from conventionally-grown potato plants contained increased number of HFs and higher hatching activity than that collected from aseptically-grown potato plants, suggesting a possible role for soil microbes in the production of HFs. Ryan & Jones (2003a) confirmed the positive effect of potato-tuber associated bacteria on HF production. Furthermore, Carroll (1995) and Cronin *et al.* (1997) isolated HF-producing rhizosphere bacteria, while Racke & Sikora (1992) demonstrated that free-living bacteria can also affect PCN hatch.

A possible second reason for the increase in *G. pallida* frequency is the inadequate control of *G. pallida* by granular carbamate nematicides, such as aldicarb (Whitehead, 1992) and oxamyl (Ambrose *et al.*, 2000), due to the slow and extended period of emergence of *G. pallida* second-stage juveniles (J<sub>2</sub>s), compared to those of *G. rostochiensis* (Evans, 1983). As a result of the short half-life of the currently available granular nematicides, such as aldicarb (Whitehead, 1992), by the time the

majority of *G. pallida* J<sub>2</sub>s hatch, the nematicides have already degraded to sub-optimal concentrations in the soil.

Recent studies have shown that inoculation of roots of potato microplants cv. Golden Wonder with Vaminoc, a commercial mixture of three *Glomus* spp. of arbuscular mycorrhizal fungi (AMF), accelerated the early hatch of *G. pallida* but did not affect the hatch of *G. rostochiensis* (Ryan *et al.*, 2000), thus eliminating the delay in *G. pallida* hatch. These studies demonstrated that mycorrhization somehow altered the *G. pallida* hatching mechanism. Ryan & Jones (2003b) found that PRL collected from the rhizosphere of Vaminoc-inoculated plants contained larger amounts of hatching factors (HFs) and hatching factor stimulants (HSs) active towards *G. pallida*, compared to the PRL from un-inoculated plants. On the other hand, AMF colonisation of potato roots stimulated root growth but at the same time increased the tolerance of the plant to *G. rostochiensis* (beneficial effect) and multiplication (detrimental effect) of *G. rostochiensis* (Ryan *et al.*, 2003). The Vaminoc-induced increase in the hatch of *G. pallida* reported by Ryan *et al.* (2000) opened up the intriguing possibility of increasing nematicidal control of *G. pallida* by inoculating potato plants with AMF. Before field trials to test this model could be carried out it would be necessary to examine the potato-PCN-AMF interaction using seed tubers, which would be the material of choice for field work. Therefore, the experimental study described in this paper was based on seed tubers, rather than on micropropagated potato plants used in previous studies by this research group (Ryan *et al.*, 2000, 2003).

The main aim of the research described here was to determine if these various effects of Vaminoc on PCN life cycle were intrinsically interconnected (i.e. the early hatch of *G. pallida* was causally related to the high multiplication of *G.*

*rostochiensis*, associated with increased root growth). This was investigated by examining the effects of the component isolates of Vaminoc on the potato-PCN interaction, with particular emphasis on PCN hatch and root growth. The Vaminoc treatment was included as a positive control with which to compare plants inoculated with single-AMF isolates and also to provide information as to how the isolates work separately and in concert.

The second objective was to analyse any AMF-associated effects on PRL hatching activity determining whether differences between AMF-inoculated and uninoculated plants in their ability to induce hatch of the two species of PCN could be attributed to altered production of HFs in the leachate, and identifying whether the component isolates of Vaminoc had better characteristics than Vaminoc with regard to HF activity of PRL towards the two PCN species. For the first time in studies of comparison of hatching activities of PRL towards PCN, the leachates were standardised on the basis of their carbon content.

## **Materials and methods**

### **Plant material**

Certified disease-free tubers (25-45 mm, CC grade) of the very late maincrop potato cultivar Golden Wonder (susceptible to both PCN species) were used in this study. The tubers were first stored for 4 weeks in cool, dry conditions (10-15°C) in order to develop strong and sturdy sprouts 2-3 cm long, after which they were planted in trays (1.2 x 0.6 x 0.2 m) containing washed quarry sand (Supamix™, Pioneer Supamix Ltd., Warwickshire, UK) at a depth of 5 cm (30 tubers per tray). While the tubers were in the trays, nutrient solution (NPK 10.0:4.4:22.5; Phostrogen Ltd., Corwen, UK), pH 6.0 at 0.5 g l<sup>-1</sup> water, was applied every 2 days from planting.



Plants were grown for 2 weeks in the trays to allow sufficient root development before AMF inoculation.

### **AMF isolates**

The AMF used were Vaminoc, a commercial mixed-isolate inoculum of three *Glomus* species, and two of these single-AMF isolates, *Glomus intraradices* (BioRize BB-E) and *Glomus mosseae* (isolate BEG 12). The mycorrhizal inocula were supplied by BioRize (Dijon, France) and contained a mixture of mycorrhizal spores, hyphae and mycorrhiza-infected sorghum root segments. The AMF propagules carrier was a calibrated calcined clay (0.5-2.5 mm).

### **Potato cyst nematodes**

Two-year-old single-generation cysts of *G. pallida* (pathotype Pa2/3) and *G. rostochiensis* (Ro1) were used (DANI, Northern Ireland). Cysts were pre-soaked on distilled water-saturated filter paper discs at 22°C for seven days prior to use.

### **In-sand inoculation with AMF**

Tubers were transplanted to 11.5 cm pots of washed quarry sand (one tuber per pot). During transplantation, tubers were separately inoculated with a granular inoculum of Vaminoc, *G. intraradices* or *G. mosseae* (1 g/plant). The AMF granules were poured into the planting holes at a depth of 5 cm in each pot. The sprouted tuber was then placed in the hole with the developing roots in contact with the AMF granules. For control plants, no AMF inoculum was placed in the planting hole. The pots were incubated under glasshouse conditions with a minimum temperature of 16°C and a 16-h photoperiod, with daylight supplemented by high-pressure sodium

vapour lamps. There were six replicate pots of each of the four treatments. The plants were each fed every 2 days with 320 ml of 0.5 g/l nutrient solution (as described above) per pot.

### **Assessment of AMF effects on plant growth**

Six weeks after shoot emergence, physiological and morphometric studies were conducted in order to determine the response of plants to mycorrhizal inoculation. Chlorophyll content, leaf area, plant height, and dry weights of leaves, shoots and roots were determined using the methodology described by Ryan *et al.* (2003).

### **Determination of AMF root colonisation**

Six weeks after shoot emergence, root samples were collected and stained with 0.05% (w/v) Trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and distilled water; Brundrett, 1994). The percentage AMF root colonisation was determined using the grid line intersect method of Giovannetti & Mosse (1980).

### **Collection of potato root leachate**

Potato root leachate was collected from each replicate pot 3 and 6 weeks after shoot emergence (Deliopoulos *et al.*, 2004). In the studies involving elemental analysis and subsequent fractionation of PRL on a Sephadex G-10 column, 100 ml of each of the six replicate leachates for each sample were pooled separately for each of the two harvest dates, concentrated by rotary evaporation at 40°C to a volume of 10 ml, re-suspended in distilled water to 20 ml and stored at 4°C until needed.

### **Hatching assay procedure**

Percentage hatch of viable eggs was determined using a modification of the microtitre plate assay method of Twomey *et al.* (1995) as employed by Byrne *et al.* (1998), using three replicates per sample with five cysts per replicate. In all *in vitro* hatching bioassays, PRL samples were diluted 1:5 with distilled water before addition to the microplates containing the pre-soaked cysts. The *in vitro* hatching tests were conducted with both PCN species on PRL samples from both collection dates (3 and 6 weeks after shoot emergence).

### **Elemental analysis**

Following rotary evaporation and re-suspension of the pooled PRL (from the six replicate pots of each sample) in distilled water to 20 ml, the re-suspended PRL material for each sample was freeze-dried and, from the dry matter produced, a sub-sample of 10 mg was analysed for carbon, hydrogen and nitrogen levels using a CE-440 Elemental Analyzer (Exeter Analytical, Massachusetts, USA), Microanalytical Laboratory, Department of Chemistry, University College Cork. Elemental analysis was conducted on PRL samples collected 3 and 6 weeks after shoot emergence.

### **Sephadex G-10 fractionation of PRL**

After elemental analysis of each sample was completed, the freeze-dried PRL was re-suspended in distilled water so that each sample contained the same concentration of organic carbon ( $1 \text{ mg ml}^{-1}$ ). An aliquot (1 ml) of this concentrate (HF preparation) was then fractionated by low pressure molecular exclusion/anion exchange liquid chromatography on a Sephadex G-10 column (2 x 38 cm) according to the method of Devine *et al.* (1996). Forty fractions (4 ml volume each) were

collected after elution of the blue dextran standard (representing the void volume). Each fraction was then assayed for hatching activity towards both PCN species.

### Data analysis

Data were analysed by parametric interaction analysis of variance (ANOVA) or unpaired t-test using the Datadesk Release 5.0 programme for Macintosh (Data Description, Inc., Ithaca, New York). Where appropriate, Fisher's Protected Least Significant Difference was used to carry out multiple comparison tests between three or more samples. In the Sephadex G-10 PCN hatching bioassays, leachate fractions which induced hatch equal to or greater than the control (water) hatch + 2 standard errors were considered to exhibit a significant hatching activity. When the hatch-active fractions were consecutive (peak zones), only the highest peak in each of these zones was considered as a HF.

### Results

Root length colonisation by all three AMF was high as shown after microscopic examination of the respective tissues. The  $F$ -ratio for AMF treatments was significant ( $F(2, 15) = 3.68, P < 0.001$ ). Vaminoc-inoculated plants exhibited the highest level of root colonisation (89.8%); significantly higher than that achieved by *G. intraradices* (66.5%) and *G. mosseae* (71.7%).

Inoculation of potato roots with all three AMF resulted in significant increases in root dry weight (Vaminoc:  $P < 0.001$ , *G. intraradices*:  $P < 0.05$ , *G. mosseae*:  $P < 0.01$ ; Table 1), compared to un-inoculated plants. Shoot dry weight was also greater in AMF-inoculated plants (except for plants inoculated with *G. intraradices*), than un-inoculated plants ( $P < 0.001$  for both Vaminoc and *G. mosseae*; Table 1). In

contrast to root and shoot dry weight, leaf dry weight was not significantly affected by mycorrhization (Table 1). No significant differences were observed between AMF-inoculated and un-inoculated plants in chlorophyll content and total leaf area produced. Similarly, shoot height was unaffected by mycorrhization (Table 1).

Assays on un-fractionated PRL showed that, within 3 weeks of shoot emergence (i.e. week 3), inoculation of potato roots with Vaminoc and *G. mosseae* (but not *G. intraradices*) had resulted in significant increases ( $P < 0.01$ ) in the hatch of viable eggs of *G. pallida* (relative to un-inoculated plants; Table 2). The mean percentage of hatched *G. pallida* eggs in leachate from non-mycorrhizal roots increased significantly ( $P < 0.001$ ; Table 2) from week 3 (31%) to week 6 (47%). On the other hand, there were no differences in *G. pallida* hatch between PRL assay dates in the AMF treatments. At week 6, the hatching response of *G. pallida* to PRL from AMF-inoculated and un-inoculated plants was more-or-less identical (mean of four treatments: 49%; Table 2). The hatching bioassays revealed that mycorrhizal inoculation of potato roots had no significant effect on the hatch of *G. rostochiensis*, unlike *G. pallida* (Table 2). Throughout the study the hatch of *G. rostochiensis* at both PRL assay dates was consistently greater than that of *G. pallida* (grand means: 82% and 48%, respectively; Table 2;  $t = 6.60$ ,  $df = 94$ ,  $P < 0.001$ ).

**Table 1. Effect of arbuscular mycorrhizal fungi (AMF) inoculation on morphological and physiological characters of potato cv. Golden Wonder 6 weeks after shoot emergence (Mean  $\pm$  SE)**

INOCULATION STATUS			
No AMF	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>
<u>Chlorophyll content (SPAD units)</u>			
42.8 $\pm$ 0.56	40.3 $\pm$ 0.32	42.1 $\pm$ 1.14	39.3 $\pm$ 1.42
<u>Total leaf area (cm<sup>2</sup>)</u>			
178.4 $\pm$ 11.62	212.1 $\pm$ 16.75	148.4 $\pm$ 27.24	176.3 $\pm$ 16.83
<u>Shoot height (cm)</u>			
17.8 $\pm$ 1.70	17.7 $\pm$ 0.92	14.5 $\pm$ 1.82	19.9 $\pm$ 0.88
<u>Leaf dry weight (g)</u>			
0.267 $\pm$ 0.043	0.359 $\pm$ 0.033	0.255 $\pm$ 0.030	0.304 $\pm$ 0.016
<u>Shoot dry weight (g)</u>			
0.515 $\pm$ 0.025	0.623*** $\pm$ 0.014	0.504 $\pm$ 0.022	0.657*** $\pm$ 0.016
<u>Root dry weight (g)</u>			
0.066 $\pm$ 0.005	0.119*** $\pm$ 0.012	0.101* $\pm$ 0.009	0.103** $\pm$ 0.008

Within a row, asterisks denote significant differences from 'No AMF' at the  $P = 0.05$  (\*),  $P = 0.01$  (\*\*) or  $P = 0.001$  levels (\*\*\*).

Plant Character	<i>F</i> -ratio <sup>a</sup>	Probability
Chlorophyll content	2.51	0.09
Total leaf area	1.85	NS
Shoot height	2.76	0.07
Leaf dry weight	2.31	NS
Shoot dry weight	15.55	<0.001
Root dry weight	6.26	<0.01

<sup>a</sup> dfs (degrees of freedom) = 3 (treatment), 20 (error), for all plant characters. NS = not significant.

**Table 2. Percentage *in vitro* hatch of (A) *Globodera pallida* and (B) *G. rostochiensis* J<sub>2</sub>s after 21 days of incubation in potato root leachate (PRL) collected 3 or 6 weeks after shoot emergence from arbuscular mycorrhizal fungi (AMF)-inoculated or un-inoculated plants**

(A)

Plant age	<i>G. pallida</i> hatch				
	No AMF	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>	<i>F</i> -ratio <sup>a</sup>
3-wk	30.9	55.0**	45.6	56.9**	4.52 ( <i>P</i> <0.05)
	<i>P</i> <0.001	NS	NS	NS	
6-wk	47.0	45.0	50.2	53.1	0.60 (NS)

(B)

Plant age	<i>G. rostochiensis</i> hatch				
	No AMF	Vaminoc	<i>G. intraradices</i>	<i>G. mosseae</i>	<i>F</i> -ratio <sup>a</sup>
3-wk	87.4	99.0	89.7	84.0	0.23 (NS)
	NS	NS	NS	NS	
6-wk	86.9	82.4	67.7	56.9	1.09 (NS)

<sup>a</sup> dfs = 3 (treatment), 20 (error). \*\* = significantly different from 'No AMF' at the *P* = 0.01 level. NS = not significant.

At 3 weeks after shoot emergence, PRL from all three mycorrhizal treatments contained less dry matter but (except for *G. intraradices*) more carbon (expressed as  $\mu\text{g ml}^{-1}$ ) than did the PRL from un-inoculated plants (Table 3). In particular, inoculation of potato roots with *G. mosseae* increased the carbon content of PRL by 37% (Table 3). In contrast, the nitrogen content of PRL collected from plants 3 weeks after shoot emergence was lower in leachate from roots inoculated with all three AMF than in leachate from non-mycorrhizal roots. A closer examination of the data (at week 3) revealed that PRL from AMF-inoculated plants (average of all three AMF) contained approximately only 62% of the nitrogen content of PRL from un-inoculated plants (Table 3). By week 6, though, the effect of AMF on carbon and

nitrogen content had changed dramatically; colonisation of potato plants by each AMF inoculum resulted in an increase in nitrogen content (Vaminoc by 22%, *G. intraradices* by 139% and *G. mosseae* by 38%) but a reduction in carbon content (Vaminoc by 44%, *G. intraradices* by 9% and *G. mosseae* by 15%), relative to that of PRL from un-inoculated plants (Table 3).

**Table 3. Composition of potato root leachate (PRL) expressed as: (I) total dry PRL matter in 20 ml freeze-dried preparation, (II) percentage C, H and N, and (III) C, H and N content ( $\mu\text{g ml}^{-1}$ ). Measurements were taken from PRL collected 3 and 6 weeks after shoot emergence.**

AMF inoculum	(I) Total dry PRL matter ( $\text{mg ml}^{-1}$ )		(II) Percentage C, H, N					
			C		H		N	
	3-wk	6-wk	3-wk	6-wk	3-wk	6-wk	3-wk	6-wk
No AMF	30.7	21.1	1.05	1.49	1.52	1.44	3.50	2.31
Vaminoc	24.5	18.6	1.54	1.17	1.19	1.26	2.68	3.20
<i>G. intraradices</i>	19.5	27.1	0.87	1.06	1.11	1.26	3.99	4.31
<i>G. mosseae</i>	11.5	15.8	3.84	1.74	2.33	1.20	4.96	4.26
(III) Content of C, H, N ( $\mu\text{g ml}^{-1}$ PRL)								
			C		H		N	
			3-wk	6-wk	3-wk	6-wk	3-wk	6-wk
No AMF			322	314	467	304	1075	487
Vaminoc			377	218	292	234	657	595
<i>G. intraradices</i>			169	287	216	341	776	1166
<i>G. mosseae</i>			442	274	268	189	570	671

Fractionation of PRL from un-inoculated plants by Sephadex G-10 chromatography revealed differences as well as similarities in the response of the two PCN species to the eluted HF<sub>s</sub> (Table 4 and Figs 1 and 2). In total, eight HF<sub>s</sub>



were resolved from the non-mycorrhizal PRL preparation with activity towards *G. pallida* and seven with activity towards *G. rostochiensis*. Four of the *G. pallida*-active HFs (D, G, I and M) were species-specific (i.e. they were absent from the *G. rostochiensis* profile), while HFs E, F and H were specific to *G. rostochiensis*. Of the four HFs common in both PCN profiles, three (HFs C, K and L) induced significantly higher hatch of *G. rostochiensis* than *G. pallida* (i.e. they were species-selective), while HF J induced similar levels of hatch in the two PCN species (species-neutral HF). The average hatching activity induced by the HFs of the leachate from non-mycorrhizal roots was significantly greater towards *G. rostochiensis* than towards *G. pallida* (23.8% and 10.4%, respectively; Table 4;  $t = 4.37$ ,  $df = 13$ ,  $P < 0.001$ ), reflecting the greater response of the former species to unfractionated PRL (Table 2).

The HF elution profile of the PRL from AMF-inoculated plants was different from that of un-inoculated plants towards each PCN species, with leachates from each AMF treatment exhibiting a distinct HF elution profile. Mycorrhization with the three AMF inocula clearly affected the activity of several existing HFs (i.e. present in the non-mycorrhizal PRL profile). The HF profiles of the four PRL preparations towards the two PCN species are illustrated in detail in Table 4 and Figs 1 and 2 (*G. pallida* and *G. rostochiensis* profiles, respectively).

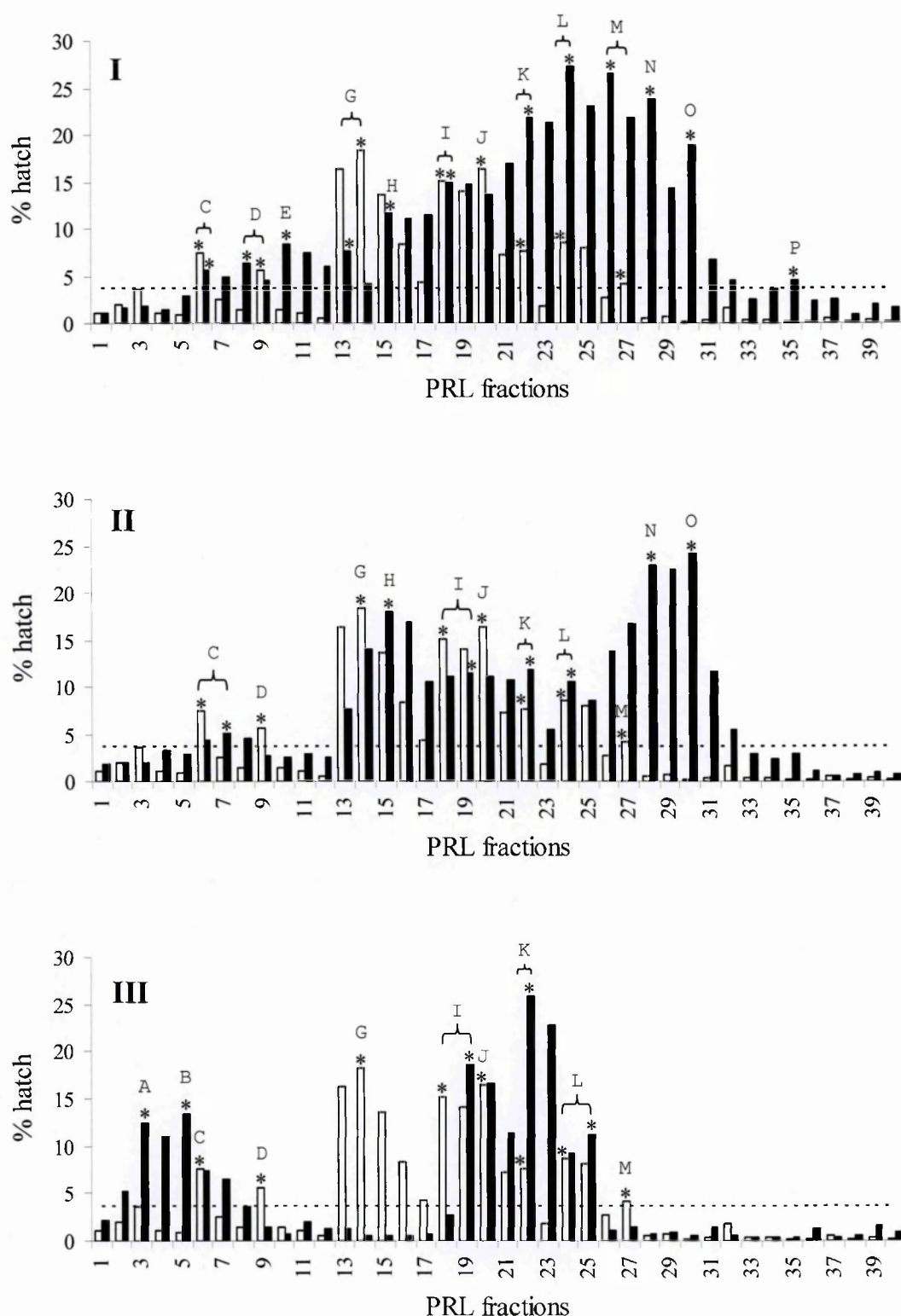
Of the 12 HFs resolved from the Vaminoc PRL preparation with activity towards *G. pallida*, five were absent from the non-mycorrhizal PRL profile (HFs E, H, N, O and P; they were novel HFs). Three of these novel HFs were also produced in the PRL from *G. intraradices*-inoculated plants but there was none detected in the PRL from *G. mosseae*-inoculated plants. Nevertheless, the leachate from *G. mosseae*-inoculated roots produced the novel HFs A and B, which were unique in the

**Table 4. Incidence of *Globodera* spp. hatching factors (HFs) in potato root leachate (PRL) from non-mycorrhizal plants (NM), or from plants inoculated with Vaminoc (M1), *Glomus intraradices* (M2) or *Glomus mosseae* (M3)**

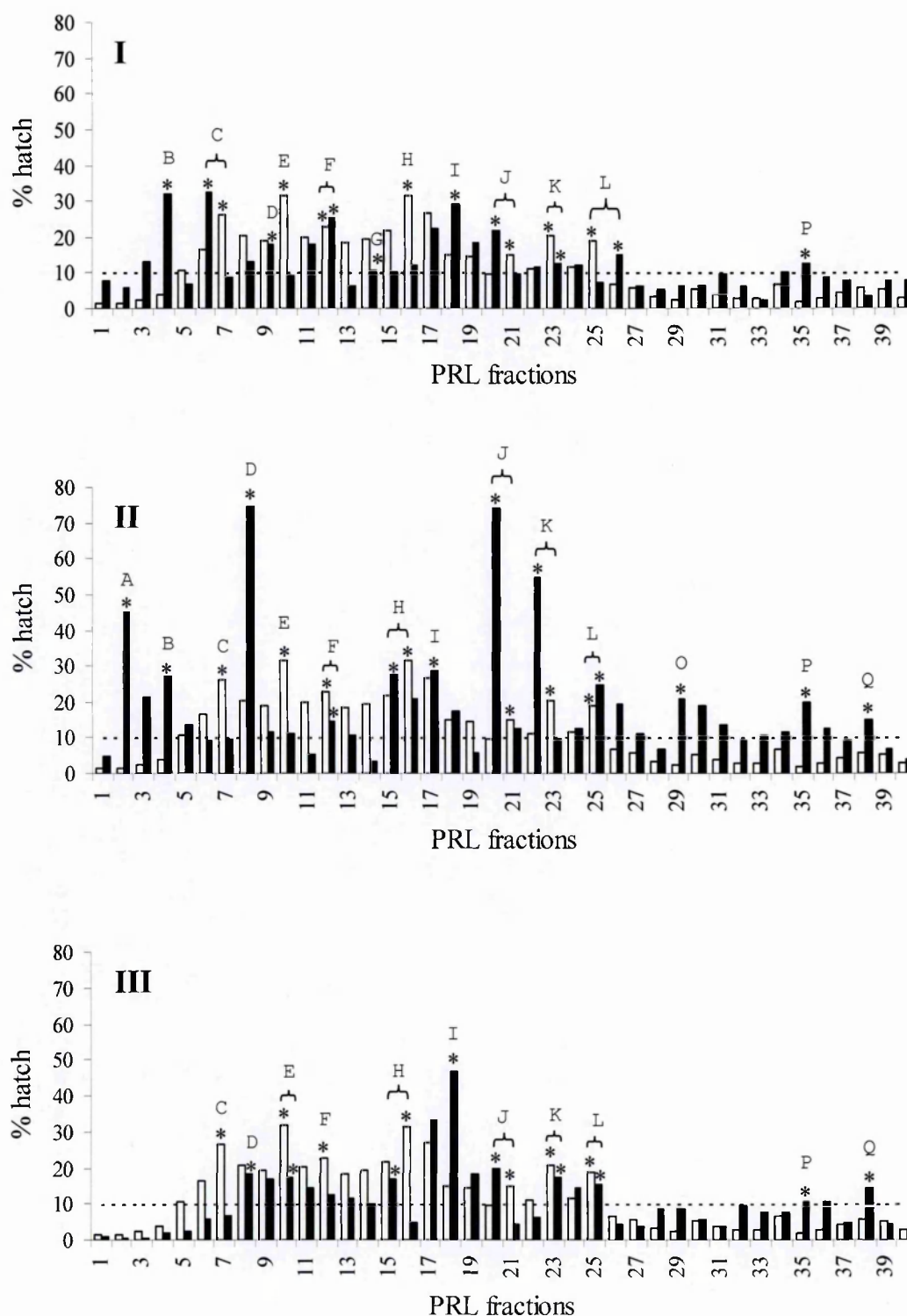
G-10 Fraction Range (Fractions)	HF	<i>G. pallida</i>				<i>G. rostochiensis</i>			
		NM	M1	M2	M3	NM	M1	M2	M3
1-5	A	-	-	-	+	-	-	+	-
	B	-	-	-	+	-	+	+	-
6-10	C	+	+	+	-	+	+	-	-
	D	+	+	-	-	-	+	+ <sup>a</sup>	+
	E	-	+	-	-	+	-	-	+
11-15	F	-	-	-	-	+	+	+	-
	G	+	+	-	-	-	+	-	-
	H	-	+	+ <sup>a</sup>	-	+ <sup>a</sup>	-	+ <sup>a</sup>	+
16-20	I	+	+	+	+	-	+	+	+
	J	+	-	-	-	+	+	+ <sup>a</sup>	+
21-25	K	+	+ <sup>a</sup>	+	+ <sup>a</sup>	+	+	+ <sup>a</sup>	+
	L	+	+ <sup>a</sup>	+	+	+	+	+	+
26-30	M	+	+ <sup>a</sup>	-	-	-	-	-	-
	N	-	+	+	-	-	-	-	-
	O	-	+	+	-	-	-	+	-
31-35	P	-	+	-	-	-	+	+	+
36-40	Q	-	-	-	-	-	-	+	+
Number of HFs		8	12	7	5	7	10	12	9
% mean hatching activity of HFs (from Figs 1 and 2)		10.4	14.8	14.9	16.3	23.8	20.9	35.6	19.6

-, HF absent; +, HF present at significant levels

+<sup>a</sup> denotes that (within each PCN species) a particular HF was significantly more active towards *G. pallida* or *G. rostochiensis* ( $P < 0.05$ ) in the selected PRL profile compared to the other PRL profiles producing the same HF



**Fig. 1.** Hatching response of *Globodera pallida* to fractions obtained by Sephadex G-10 fractionation of potato root leachate (PRL) collected 3 weeks after shoot emergence from non-mycorrhizal (open bars, □) and mycorrhizal (solid bars, ■) plants; comparing non-mycorrhizal with: (I) Vaminoc-, (II) *Glomus intraradices*- and (III) *Glomus mosseae*-colonised potato plants. Broken horizontal line refers to hatch in the presence of water + 2 standard errors. Asterisks and letters indicate peaks of significant hatching factor (HF) activity towards *G. pallida*.



**Fig. 2.** Hatching response of *Globodera rostochiensis* to fractions obtained by Sephadex G-10 fractionation of potato root leachate (PRL) collected 3 weeks after shoot emergence from non-mycorrhizal (open bars, □) and mycorrhizal (solid bars, ■) plants; comparing non-mycorrhizal with: (I) Vaminoc-, (II) *Glomus intraradices*- and (III) *Glomus mosseae*-colonised potato plants. Broken horizontal line refers to hatch in the presence of water + 2 standard errors. Asterisks and letters indicate peaks of significant hatching factor (HF) activity towards *G. rostochiensis*.

*G. mosseae* profile. There was a marked absence of significant hatching activity towards *G. pallida* in the *G. mosseae* PRL profile at elution volumes corresponding to fractions 8-18 and 26-40, resulting in down regulation of a number of HFs (e.g. D, G and M). In addition to generating novel HFs, inoculation of potato roots with Vaminoc also increased significantly the activities of three HFs (K, L and M), a phenomenon not observed, though, with the single-AMF isolates, the only exception being HF K in the PRL profile of *G. mosseae*-inoculated plants. The only HF that mycorrhization of potato plants with the three AMF inocula had no effect on its activity towards *G. pallida* was HF I.

Mycorrhization of the potato plants with Vaminoc and with the two single-AMF isolates *G. intraradices* and *G. mosseae* resulted in the resolution of eight novel HFs (A, B, D, G, I, O, P and Q) with activity towards *G. rostochiensis*. Of the five, in total, HFs present in the Vaminoc PRL profile, four (B, D, I, P) were also eluted from the PRL of *G. intraradices*-inoculated plants, and three were also present in the PRL of *G. mosseae*-inoculated plants. The inoculum that induced the greatest activity towards *G. rostochiensis* in the fractionated PRL was *G. intraradices*. In particular, HFs produced in the PRL from *G. intraradices*-inoculated plants stimulated the hatch of an average 35.6% of *G. rostochiensis* eggs (Table 4); this was significantly greater than that caused by the PRL from the other two AMF treatments (but not from the un-inoculated controls).

As for *G. pallida*, the HF profile of each AMF treatment towards *G. rostochiensis* was different from that of un-inoculated plants, but this time there was markedly less variation among the three mycorrhizal PRL profiles with regard to the number of HFs produced. Six HFs active towards *G. rostochiensis* were common in the three mycorrhizal PRL profiles (HFs D, I, J, K, L and P) compared to only three

such HFs in the *G. pallida* HF profile (HFs I, K, L). Furthermore, comparison of the *G. rostochiensis* hatch response to fractions of PRL from *G. intraradices*-inoculated and *G. mosseae*-inoculated plants revealed that eight HFs (D, H, I, J, K, L, P and Q) were common in the two profiles compared to less than twice that number for *G. pallida* (three HFs: I, K and L). The two most active *G. rostochiensis* HFs were resolved from the PRL of *G. intraradices*-inoculated plants (HFs D and J), with over 70% hatched J<sub>2</sub>s per cyst. In contrast with *G. rostochiensis*, none of the HFs with activity towards *G. pallida* stimulated hatch in excess of 30%. There were also major differences in the hatch responses between the two PCN species to the HFs resolved from each of the three mycorrhizal PRL preparations. For example, of the 15 HFs produced in total in the PRL from Vaminoc-inoculated plants with activity towards the two PCN species, only three (HFs G, K and P) induced similar hatch responses in the two species (species-neutral HFs). Several HFs from the Vaminoc PRL preparation exhibited PCN species-specificity (e.g. *G. pallida*-specific: HFs M, N and O; *G. rostochiensis*-specific: HFs B, F and J).

## Discussion

The results from the PCN hatching bioassays in un-fractionated PRL demonstrated that the component isolates of Vaminoc did not exhibit better characteristics than Vaminoc with regard to the effect on PCN hatch as, in the majority of the cases, the effect was similar. However, one of the isolates, *G. intraradices*, failed to induce a significant increase in the early hatch of *G. pallida*, unlike Vaminoc and *G. mosseae*. On the other hand, both isolates increased root growth, as did Vaminoc, with no significant differences among the three AMF. These findings suggest that the effects of Vaminoc on the PCN life cycle reported by

Ryan *et al.* (2003) (i.e. increased *G. pallida* hatch and *G. rostochiensis* multiplication) were interconnected; if they were not it would have been expected one component isolate to cause the hatch effect and the other one the root growth and hence, the tolerance and multiplication effects. Consequently, it seems unlikely that replacement of the mixed-isolate Vaminoc inoculum with individual AMF isolates in future studies of potato-PCN-AMF interaction would give any better results with respect to the proposed use of AMF in PCN control.

In order to determine whether the single-AMF isolates had different effects than Vaminoc on other aspects of the potato-PCN interaction, the effectiveness of each single *Glomus* isolate was individually compared with that of Vaminoc on all parameters assessed. These comparisons revealed that on only two occasions (percentage root length colonisation and number of *G. pallida*-active HF's produced in PRL) were both the single-AMF isolates less effective than Vaminoc. These were also the only cases where *G. mosseae* departed significantly from the Vaminoc effect. On the contrary, other differences between *G. intraradices* and Vaminoc with regard to their effects on the potato-PCN interaction were apparent. For example, inoculation of potato roots with Vaminoc increased the carbon content of PRL at week 3, compared to un-inoculated plants (*G. intraradices* almost halved it) and it also increased the shoot dry weight of plants at week 6 (*G. intraradices*-inoculated plants exhibited similar shoot dry weight with un-inoculated plants). While the result of AMF inoculation of potato on PCN hatch is consistent, the effects on plant growth appear to be more variable. For example, Vaminoc increased shoot dry weight of the late maincrop cv. Golden Wonder in the present study, while previously, under the same growing conditions, it has been shown to reduce shoot dry weight of plants in the early maincrop cv. Maris Piper (Deliopoulos *et al.*, 2004), suggesting that the

effect of AMF on plant growth could be associated with the maturity level of the cultivar used.

The *in vitro* hatch results confirmed earlier findings that, in the absence of AMF, *G. pallida* hatch was slower than that of *G. rostochiensis* (Evans, 1983; Whitehead, 1992; Ryan *et al.*, 2000; Deliopoulos *et al.*, 2004) and that inoculation of potato with Vaminoc increased the early hatch of *G. pallida* but not of *G. rostochiensis* (Ryan *et al.*, 2000; Ryan *et al.*, 2003; Deliopoulos *et al.*, 2004). The consistency of these effects between experiments supports the veracity of these findings. Standardisation of root leachates by carbon content allowed direct quantitative and qualitative comparisons of hatching activities between the four PRL profiles towards the two PCN species. It is the first time in studies of this nature that HF profiles of PRL are corrected for differences in carbon content. An equally effective method for comparing PRL hatching activities is by standardising the leachates on the basis of dry weight (Devine & Jones, 2001). The advantage of these techniques of leachate standardisation compared to other approaches (e.g. by root weight or root volume; Turner & Stone, 1981) is the reduction of differences in root development characteristics; such differences could have a large effect on the capacity of roots to generate leachate, thus making direct comparison of PRL hatching activities difficult.

Fractionation of the PRL preparations on Sephadex G-10 column revealed that there were multiple effects of AMF on HF profiles which were either quantitative or qualitative, mainly up-regulation but also down-regulation. For example, in the profile of the *G. pallida* response to PRL fractions, inoculation with Vaminoc and *G. mosseae* increased the activity of HF K (quantitative effect), while inoculation with *G. mosseae* resulted in the resolution of the novel HF B (qualitative effect). The



existence of PCN species-specific, species-selective and in a few cases, species-neutral HFs (in both the non-mycorrhizal and the three mycorrhizal PRL profiles) supports previous reports of this research group that the two PCN species respond differently to the majority of the HFs produced in host root leachate (Byrne *et al.*, 2001; Devine & Jones, 2001; Ryan & Jones, 2003b).

The significantly greater hatch of *G. pallida* in the un-fractionated PRL from Vaminoc-inoculated compared to un-inoculated plants was mirrored in the fractionated PRL, where not only were several novel HFs identified in the Vaminoc PRL profile but also significant increases in the activity of some existing HFs were observed. In comparison with the mixed-AMF inoculum, PRL collected from plants inoculated with the two single-AMF isolates contained markedly fewer HFs. This result indicates that the stimulatory effect of *G. mosseae* on the hatch of *G. pallida* in the un-fractionated PRL was not due to increased production of HFs per unit of carbon in the leachate. In the current experiment, although both the single-AMF isolates had stimulatory effects on root growth, only *G. mosseae* had a similar effect on *G. pallida* hatch. Increased root size could be due to increased root length (no effect on HF production) and/or to increased root branching, resulting in more root tips and hence, increased HF production (Rawsthorne & Brodie, 1986). According to that, an hypothesis can be proposed that the *G. mosseae*-induced stimulation of root growth was due to increased root branching, while in the case of *G. intraradices* to an increase in root length. To investigate this possibility the root systems of plants inoculated with these AMF inocula will need to be examined and compared with regard to their morphological characteristics (number of lateral roots and root tips present, measurement of root length); similar comparisons should also be conducted between AMF-inoculated and un-inoculated plants.

possibility is that mycorrhizal fungi synthesise these HFs themselves and release them in the leachate. Recent studies (Ryan *et al.*, 2000; Deliopoulos *et al.*, 2004) showed that the mixed-isolate Vaminoc was unable to cause any increase in the in-soil hatch of either PCN species when inoculated onto roots of PCN non-host (strawberry and wheat) plants, suggesting that AMF induce the production of novel fungal HFs only in the presence of potato roots. Research on the chemical nature of HFs has shown that they are molecules of terpenoid nature (Mulder *et al.*, 1992; Jones *et al.*, 1998). Their separation from root leachate preparations by gel permeation chromatography is based on both size and weak anion exchange (Devine & Jones, 2000). The study of Devine & Jones (2000) showed that the multiple HFs produced in PRL had all the same molecular weight (530.5 Da) but they differed in their daughter ions, indicating that there are minor structural differences among HFs. Nevertheless, as yet, the chemical structure of these HFs has not been identified. Mulder *et al.* (1992) presented the formula  $C_{27}H_{30}O_9$  for a PCN HF isolated from root leachate of tomato plants. Schenk *et al.* (1999) published the chemical structure of this HF, which they named solanoeclepin A. Since potato HFs have the same molecular mass, only purification of mycorrhizal-specific PCN HFs and identification of their molecular weight would elucidate the origin (potato- or fungal-derived) of these novel HFs.

The high levels of potato root length colonisation by Vaminoc, *G. intraradices* and *G. mosseae* observed in this study confirmed that these selected commercial AMF inocula (Deliopoulos *et al.*, 2004) are highly successful potato root colonisers. Gaur & Adholeya (2000) also reported that inoculation of potato roots with a mixed culture of endomycorrhizal fungi resulted in high root length colonisation (approximately 65%). The high percentage of root colonisation by all three AMF

Although the *in vitro* hatch of *G. rostochiensis* was similar from AMF-inoculated and un-inoculated plants in the un-fractionated PRL collected 3 weeks after shoot emergence, fractionation of the four PRL preparations on Sephadex G-10 column revealed that root leachates collected from AMF-inoculated plants of all three treatments contained higher number of HFs active towards *G. rostochiensis* compared to the PRL from un-inoculated plants. The reason for this effect is not known, although it was also observed in the study of Ryan & Jones (2003b). Within 3 weeks of shoot emergence the hatch of *G. rostochiensis* was very high (84 to 99%) and thus it had possibly peaked, therefore, in contrast with *G. pallida*, increase in HF production had no effect on total *in vitro* hatch. The observation that hatch of *G. rostochiensis* in un-fractionated PRL collected from un-inoculated and AMF-inoculated plants (all treatments) 3 weeks after shoot emergence exceeded 80% was in agreement with the report of LaMondia & Brodie (1986) in that the majority of *G. rostochiensis* J<sub>2</sub>s which invaded the potato root hatched within the first 3 weeks from planting. Overall, in comparison with *G. pallida*, there was less variation in the quantity of HFs in the PRL profiles of the three AMF treatments.

Analysis of individual fractions collected after Sephadex G-10 chromatography of the four PRL preparations showed that the HF elution profiles of *G. intraradices*-inoculated and *G. mosseae*-inoculated plants differed from the profiles of Vaminoc-inoculated and un-inoculated plants in the ratio of *G. rostochiensis* : *G. pallida* specific HFs (7:2 and 6:2 compared to 3:5 and 3:4, respectively), demonstrating a preference for *G. rostochiensis* of the HFs produced in the *G. intraradices* and *G. mosseae* PRL profiles.

The origin of these *G. pallida* and/or *G. rostochiensis* hatch-stimulating chemicals resolved from the PRL of AMF-inoculated plants is not clear. One

used in this study could be attributed to the fact that AMF inoculation was performed on sprouted tubers with a sufficiently developed root system. Potato plants grown from AMF-inoculated sprouted tubers would normally exhibit higher levels of mycorrhizal colonisation than would do plants from un-sprouted tubers, presumably because of the delay in generating colonisable tissue in the latter case. The importance of a sufficiently developed root system prior to mycorrhizal inoculation is often emphasised in the literature as a key for optimum root colonisation by mycorrhizae (e.g. Vestberg & Estaún, 1994; Mark & Cassells, 1996).

Ryan *et al.* (2003) found that the effect of Vaminoc on hatch was associated with increased root growth. Similar results were obtained here with all three AMF increasing the root dry weight of plants. The stimulatory effect of mycorrhization on root growth is according to Atkinson *et al.* (1994) the most frequent consequence of AMF colonisation. Three weeks after shoot emergence (when the stimulatory effect on *G. pallida* hatch was observed), potato plants inoculated with all three AMF contained lower PRL dry matter than the un-inoculated plants, but (except for *G. intraradices*, the least hatch active AMF isolate) increased PRL carbon content. These results suggest that at least part of the hatch-stimulatory AMF effect could be due to an increase in the carbon content of the root leachate (associated with increased root dry matter). The negative association between carbon and nitrogen content of PRL, both between AMF treatments (Vaminoc and *G. mosseae* in particular) and between assay dates, was consistent. These results are also in agreement with the observations of Ryan & Jones (2003b) who found that PRL collected from Vaminoc-inoculated plants within the first 4 weeks from planting contained 20% more carbon but 48% less nitrogen than the leachate collected from un-inoculated roots.

The result of mycorrhizal colonisation of potato roots on plant growth was, in the main, similar among the three AMF treatments. Only with regard to shoot dry weight was the response of potato plants to inoculation with the three AMF preparations dissimilar. This observation, however, was not unexpected since inconsistent effects of AMF on plant growth are well known from many other AMF associations and such examples are in abundance in the mycorrhizal literature. As Johnson & Pfleger (1992) have emphasised, an effective AMF root coloniser does not always imply plant growth promotion. Examination of leaf characters from AMF-inoculated and un-inoculated plants (leaf area, chlorophyll content, dry weight) did not reveal any significant AMF effect, but this could be simply due to the early stage of plant growth (6 weeks after first shoots emerged) at which the plants were harvested, by which time the stimulatory effects of AMF on root and shoot growth had not yet become apparent in the leaves. Another scenario that could explain the lack of a significant effect of AMF on the leaves is a slowdown in plant metabolic activity after the nutrients have been transferred to the shoot of the plant from the root.

The AMF-induced elimination of the delay of *G. pallida* hatch could increase the effectiveness of nematicides under AMF-inoculated plants via synchronisation of maximum nematode hatch with optimum nematicide concentration. If the model described in this communication proves effective under field conditions, it would offer the perspective on an integrated (chemical-biological) control of *G. pallida*. The next step in this research would be to investigate the interaction between potato plants, *G. pallida*, AMF and the nematicide aldicarb under conditions of close proximity to the field situation.

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## References

- AMBROSE, E., HAYDOCK, P.P.J. & WILCOX, A. (2000). Degradation of the nematicide oxamyl in field conditions. *Aspects of Applied Biology* 59, 41-51.
- ATKINSON, D., BERTA, G. & HOOKER, J.E. (1994). Impact of mycorrhizal colonisation on root architecture, root longevity and the formation of growth regulators. In: Gianinazzi, S. & Schüepp, H. (Eds.). *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*. Basel, Switzerland, Birkhäuser Verlag, pp. 89-99.
- BRUNDRETT, M. (1994). Clearing and staining mycorrhizal roots. In: Brundrett, M., Melville, L. & Peterson, L. (Eds.). *Ninth North American Conference on Mycorrhizae*. University of Guelph, Ontario, Canada, Mycologue Publications, pp. 42-46.
- BYRNE, J., MAHER, N., & JONES, P.W. (2001). Comparative responses of *Globodera rostochiensis* and *G. pallida* to hatching chemicals. *Journal of Nematology* 33, 195-202.
- BYRNE, J., TWOMEY, U., MAHER, N., DEVINE, K.J. & JONES, P.W. (1998). Detection of hatching inhibitors and hatching factor stimulants for golden potato

- cyst nematode, *Globodera rostochiensis*, in potato root leachate. *Annals of Applied Biology* 132, 463-472.
- CARROLL, M. (1995). Characterisation of rhizosphere bacterial isolates which affect the hatch of potato cyst nematodes. MSc thesis, The National University of Ireland, Cork, Ireland, 70 pp.
- CRONIN, D., MOENNE-LOCCOZ, Y., FENTON, A., DUNNE, C.C., DOWLING, D.N. & O'GARA, F. (1997). Role of 2,4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. *Applied and Environmental Microbiology* 6, 1357-1361.
- DELIOPOULOS, T., HAYDOCK, P.P.J. & JONES, P.W. (2004). Variation in plant growth and *in vitro* hatching activity towards potato cyst nematodes in the response of different potato cultivars to inoculation with arbuscular mycorrhizal fungus isolates. *Annals of Applied Biology*, in press.
- DEVINE, K.J., BYRNE, J., MAHER, N. & JONES, P.W. (1996). Resolution of natural hatching factors for the golden potato cyst nematode, *Globodera rostochiensis*. *Annals of Applied Biology* 129, 323-334.
- DEVINE, K.J. & JONES, P.W. (2000). Purification and partial characterisation of hatching factors for the potato cyst nematode *Globodera rostochiensis* from potato root leachate. *Nematology* 2, 231-236.
- DEVINE, K.J. & JONES, P.W. (2001). Potato cyst nematode hatching activity and hatching factors in inter-specific *Solanum* hybrids. *Nematology* 3, 141-149.
- EVANS, K. (1983). Hatching of potato cyst nematodes in root diffusates collected from twenty-five potato cultivars. *Crop Protection* 2, 97-103.

- GAUR, A. & ADHOLEYA, A. (2000). Response of three vegetable crops to VAM fungal inoculation in nutrient deficient soils amended with organic matter. *Symbiosis* 10, 19-31.
- GIOVANNETTI, M. & MOSSE, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular infection in roots. *New Phytologist* 84, 489-500.
- JOHNSON, N.C. & PFLEGER, F.L. (1992). Vesicular-arbuscular mycorrhizae and cultural stresses. In: Bethlenfalvay, G.J. & Linderman, R.G. (Eds). *Mycorrhizae in Sustainable Agriculture*. Madison, Wisconsin, USA, ASA special publication No. 54, American Society of Agronomy, Inc., pp. 71-99.
- JONES, P.W., TYLKA, G.L. & PERRY, R.N. (1998). Hatching. In: Perry, R.N. & Wright, D.J. (Eds). *The Physiology and Biochemistry of Free-Living and Plant-Parasitic Nematodes*. Wallingford, UK, CAB International, pp. 181-212.
- LAMONDIA, J.A. & BRODIE, B.B. (1986). The effects of potato trap crops and fallow on decline of *Globodera rostochiensis*. *Annals of Applied Biology* 108, 347-352.
- MARK, G.L. & CASSELLS, A.C. (1996). Genotype-dependence in the interaction between *Glomus fistulosum*, *Phytophthora fragariae* and the wild strawberry (*Fragaria vesca*). *Plant and Soil* 185, 233-239.
- MINNIS, S.T., HAYDOCK, P.P.J., IBRAHIM, S.K., GROVE, I.G., EVANS, K. & RUSSELL, M.A. (2002). Potato cyst nematodes in England and Wales - Occurrence and distribution. *Annals of Applied Biology* 140, 187-195.
- MULDER, J.G., DIEPHENHORST, P., PLIEGER, P. & BRÜGGEMANN-ROTGANS, I.E.M. (1992). Hatching agent for the potato cyst nematode. *Patent Application No. PCT/NL92/00126*.
- RACKE, J. & SIKORA, R.A. (1992). Influence of the plant health promoting rhizobacteria *Agrobacterium radiobacter* and *Bacillus sphaericus* on *G. pallida*



- root infection and subsequent plant growth. *Journal of Phytopathology* 134, 198-208.
- RAWSTHORNE, D. & BRODIE, B.B. (1986). Relationship between root growth of potato, root diffusate production, and hatching of *Globodera rostochiensis*. *Journal of Nematology* 18, 379-384.
- RYAN, N.A., DELIOPOULOS, T., JONES, P.W. & HAYDOCK, P.P.J. (2003). Effects of a mixed-isolate mycorrhizal inoculum on the potato - potato cyst nematode interaction. *Annals of Applied Biology* 143, 111-119.
- RYAN, N.A., DUFFY, E.M., CASSELLS, A.C. & JONES, P.W. (2000). The effect of mycorrhizal fungi on the hatch of potato cyst nematodes. *Applied Soil Ecology* 15, 233-240.
- RYAN, N.A. & JONES, P.W. (2003a). Effect of tuber-borne micro-organisms on hatching activity of potato root leachate towards potato cyst nematodes. *Nematology* 5, 55-63.
- RYAN, N.A. & JONES, P.W. (2003b). The effect of mycorrhization of potato roots on the hatching chemicals active towards the two potato cyst nematode species, *Globodera pallida* and *Globodera rostochiensis*. *Nematology*, in press.
- SCHENK, H., DRISSEN, R.A.L., DE GELDER, R., GOUBITZ, K., NIEBOER, H., BRÜGGEMANN-ROTGANS, I.E.M. & DIEPHENHORST, P. (1999). Elucidation of the structure of Solanoecepin A, a natural hatching factor of potato and tomato cyst nematodes, by single-crystal X-ray diffraction. *Croatica Chemica Acta* 72, 593-606.
- TWOMEY, U., RAFTERY, T., DEVINE, K.J. & JONES, P.W. (1995). An improved procedure for assaying hatching activity towards *Globodera rostochiensis*. *Nematologica* 41, 258-268.

- TURNER, S.J. & STONE, A.R. (1981). Hatching of potato cyst nematode (*Globodera rostochiensis* and *G. pallida*) in root exudates of *Solanum vernei* hybrids. *Nematologica* 27, 315-318.
- VESTBERG, M. & ESTAÚN, V. (1994). Micropropagated plants, an opportunity to positively manage mycorrhizal activities. In: Gianinazzi, S. & Schüepp, H. (Eds). *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*. Basel, Switzerland, Birkhäuser Verlag, pp. 217-226.
- WHITEHEAD, A.G. (1992). Emergence of juvenile potato cyst nematodes *Globodera rostochiensis* and *G. pallida* and the control of *G. pallida*. *Annals of Applied Biology* 120, 471-486.

## **Chapter E**

### **Interaction between arbuscular mycorrhizal fungi and the nematicide aldicarb on potato cyst nematode (*Globodera pallida*) hatch, nematode development and yield of potatoes**

By T DELIOPOULOS, P HAYDOCK and P W JONES

All the research described in this chapter was carried out by TD

## Summary

The effects on hatch and multiplication of the potato cyst nematode *Globodera pallida* (Stone), and root dry weight, shoot dry weight and fresh tuber yield in response to inoculation with a mixed-isolate (Vaminoc) and three single isolates (*Glomus intraradices*, *Glomus mosseae* and *Glomus dussii*; components of Vaminoc) of arbuscular mycorrhizal fungi (AMF) were assessed on potato (*Solanum tuberosum* L.) cultivar Golden Wonder in the presence and absence of the nematicide aldicarb (Temik 10G, Bayer CropScience Ltd) in a pot experiment. Mycorrhization of potato roots had a stimulatory effect on the early hatch of *G. pallida*, particularly after 2 wk (in the presence of nematicide) or 4 wk (in absence). Root length colonisation by AMF was unaffected by the application of aldicarb. Roots of PCN-infested plants exhibited reduced levels of mycorrhizal colonisation compared to non-PCN-infested plants but this effect was significant only in *G. intraradices*-inoculated plants. The degree of mycorrhizal infection in roots was significantly higher in plants inoculated with the Vaminoc mixture than in plants inoculated with the Vaminoc component isolates. Mycorrhization of potato roots with each of the four AMF preparations caused a significant reduction in the multiplication of *G. pallida* in both the presence and absence of aldicarb. In the presence of aldicarb, AMF-inoculated plants exhibited only 57% of the PCN population size (viable eggs g<sup>-1</sup> soil) of the non-mycorrhizal plants; in the absence of aldicarb the respective value was 42%. The AMF isolates used differed in their ability to produce a plant growth response (expressed as root dry weight, shoot dry weight or total dry biomass) and to affect tuber yield. In this regard, the single *Glomus* isolates enhanced plant growth and improved fresh tuber yield, while Vaminoc had in most cases no effect. The results are discussed in terms of developing a novel method for controlling *G. pallida*.

population increases *via* increased granular nematicide effectiveness by applying AMF as part of an integrated *G. pallida* control strategy.

**Key words:** *Globodera pallida*, arbuscular mycorrhizal fungi, aldicarb, hatch, multiplication, root length colonisation, plant growth, yield

## Introduction

Potato cyst nematodes (PCN) *Globodera pallida* (Stone) and *Globodera rostochiensis* (Woll.) are the most serious pests of the potato (*Solanum tuberosum* L.) crop in many potato-growing regions. In the UK, in particular, the annual yield losses in total ware potato production due to the damage caused by PCN has been estimated to be about 9% (Evans & Brodie, 1980).

The most widely used method of chemical control for PCN in the UK involves application of the granular carbamate nematicides Temik (aldicarb; Bayer) and Vydate (oxamyl; DuPont) at planting. Under susceptible potato cultivars, aldicarb and oxamyl have been shown to be very effective in controlling *G. rostochiensis* population increase but to have only a limited effect on the increase of *G. pallida* (Whitehead, 1992). The reduced effectiveness of the available granular nematicides as well as of crop rotation against *G. pallida*, compared to *G. rostochiensis*, has been attributed to differences in the life cycle and in the hatching behaviour, in particular, between the two PCN species. The extended period of emergence of *G. pallida* second-stage juveniles (J<sub>2</sub>s) with a later annual peak of hatching activity than *G. rostochiensis* during the growing season, combined with the short half-lives of aldicarb and oxamyl, means that by the time the majority of J<sub>2</sub>s will hatch and

become active in the soil, the nematicide may have degraded and therefore be ineffective (Whitehead, 1992).

In contrast with *G. rostochiensis*, there are not currently any potato cultivars with full resistance against *G. pallida*; only partial resistance is available against *G. pallida* in commercial potato cultivars (e.g. cvs Sante and Nadine; Whitehead, 1997). In addition to that, the widespread and repeated use of *G. rostochiensis*-resistant cultivars (containing the H1 gene) in fields with mixed populations of PCN allowed the *G. pallida* population levels to increase in the UK. As a result, a recent survey revealed that 92% of the PCN-infested land in the UK currently contains *G. pallida* (Minnis *et al.*, 2002).

*In vitro*, hatching of J<sub>2</sub>s from encysted eggs in both species of PCN occurs in response to multiple hatching factors (HFs) produced in host-specific root leachate (Perry, 1989; Devine *et al.*, 1996; Jones *et al.*, 1998). However, field and pot trials have demonstrated that, in soil, the hatch of PCN may be affected by the activity of soil micro-organisms. Tsutsumi (1976) observed that the spontaneous hatch of PCN in the field was very high for up to 100 days after the removal of the potato crop; these seasonal flushes of PCN hatch in fallow soil could be the result of microbial involvement in the production of HFs although the author did not suggest that. Devine *et al.* (1996) reported that potato root leachate (PRL) collected from aseptically-grown plants contained lower hatching activity towards *G. rostochiensis* and only a proportion of the HFs found in PRL from non-sterile-grown plants, suggesting that several of the (late-eluting) HFs present in conventionally-produced PRL may be microbial in origin. Carroll (1995) isolated HF-producing rhizobacteria with absolute specificity towards *G. pallida* or *G. rostochiensis*, while Ryan & Jones (2003a) demonstrated that root leachate collected from potato microplants grown

with tuber bacteria contained more HFs and hatching factor stimulants (HSs, hatch-neutral chemicals which stimulate HF-induced hatch without having any effect in isolation on hatching; Byrne *et al.*, 1998) but fewer hatching inhibitors (HIs, chemicals which inhibit the HF-induced hatch of PCN; Byrne *et al.*, 1998) than leachate from aseptically-grown potato microplants. Recent research that has been focused on the effects of arbuscular mycorrhizal fungi (AMF) on the hatch of PCN, have provided increasing evidence for a role for mycorrhizal HFs in the PCN hatching mechanism. Mycorrhizal-induced stimulation of *G. pallida* hatch, but not of *G. rostochiensis*, has been shown so far with Vaminoc (a mixed-isolate inoculum consisting of three *Glomus* spp.) (Ryan *et al.*, 2000; Ryan & Jones, 2003b; Deliopoulos *et al.*, 2004) and with the single AMF isolates *Glomus intraradices* and *Glomus mosseae* (Deliopoulos *et al.*, 2004) in *in vitro* PRL and in-sand studies.

Since the limited effectiveness of granular nematicides in controlling *G. pallida* increase in the field has been associated with the delayed hatch of this species, the hypothesis is that an AMF-induced acceleration of the early hatch of *G. pallida* should increase the effectiveness of nematicides by controlling the *G. pallida* population increase. This study was designed to test this hypothesis and to test the compatibility of AMF isolates with granular nematicides and it is the first report for non-sterile soil studies on the potato-PCN-AMF-nematicide interaction. In addition to the Vaminoc mixture, the three component isolates of Vaminoc, i.e. *G. intraradices*, *G. mosseae* and *G. dussii*, were also tested in order to determine whether AMF-inoculated plants exhibit better characteristics with regard to *G. pallida* hatch, *G. pallida* population increase and tuber yield, compared to non-mycorrhizal plants.

## Materials and Methods

### Plant material

Certified seed potatoes (25-45 mm, CC grade) of the maincrop cultivar Golden Wonder were used. Golden Wonder is a cultivar of very late maturity with susceptibility to both PCN species. The tubers were chitted for 3 wk at 20°C until they produced sprouts 2-3 cm long.

### Potato cyst nematodes

Single-generation two-year-old cysts of *Globodera pallida* (pathotype Pa2/3) were used (courtesy of C. Fleming, DARDNI, Northern Ireland). The cysts were pre-soaked on distilled water-saturated discs of filter paper at 22°C for 7 days prior to use.

### Arbuscular mycorrhizal fungi

The AMF isolates tested included Vaminoc (also known as Endorize-Mix), which is a mixed-isolate AMF inoculum, and three single-isolate AMF preparations; *Glomus intraradices* (BioRize BB-E), *Glomus mosseae* (isolate BEG 12) and *Glomus dussii* (BioRize BB-Sc1). The Vaminoc mixture consists of these three *Glomus* species (Dr B. Blal, BioRize). All AMF isolates were commercially produced and supplied by BioRize (Dijon, France).

### Treatments

The sprouted tubers were each pre-inoculated with 5 g of one of the four granular AMF isolates (placed in the bottom of the planting hole) in trays (1.2 x 0.6 x 0.2 m) containing a loam/sandy loam soil (51% sand, 33% silt, 13% clay), pH 6.0,



3% organic matter, at a depth of 5 cm (30 tubers per tray). For control plants, no AMF inoculum was placed into the planting hole. The concentration of phosphorus (P) in the soil was 23.3 ppm, while the potassium (K), magnesium (Mg) and calcium (Ca) content of the soil were 206 ppm, 75 ppm and 1365 ppm, respectively. Soil analysis was conducted by Johnstown Castle Labs (Wexford, Ireland). The soil had been removed from a fallow field planted the previous year to barley plants and did not contain any indigenous PCN. The sprouted tubers were maintained for 2 wk in the trays to induce rooting and AMF colonisation and were fertilised at planting and every 2 days thereafter with nutrient solution (NPK 10.0:4.4:22.5; Phostrogen Ltd., Corwen, UK), pH 6.0 at a rate of 0.5 g l<sup>-1</sup> water per tray.

Prior to transplanting, 17.5 cm pots containing the loam/sandy loam soil were treated with nematicide and PCN cysts (90 pots), nematicide only (90), PCN cysts only (90) or neither PCN nor nematicide (90). The nematicide used was aldicarb (Temik 10G, 10% a.i. w/w granules, Bayer CropScience Ltd). Aldicarb granules were mixed with sufficient amount of soil in a cement mixer to give a soil concentration of 200 mg aldicarb pot<sup>-1</sup> (calculated per area using the top diameter of the pot, 27.5 cm, and the commercial field rate of Temik 10G, 33.6 kg ha<sup>-1</sup>) and the treated soil was then placed in the pots to a depth of approximately 15 cm. Appropriate safety guidelines were followed for the handling of treated soil or plants. For pots containing PCN, the inoculation procedure followed the methodology of Byrne *et al.* (1998), with sealed cotton muslin sachets (5 cm x 5 cm), each containing 60 pre-soaked cysts of *G. pallida*, placed in each pot at a depth of 2 cm below the root system. Tubers from the mycorrhizal treatments were separately inoculated with 5 g of Vaminoc, *G. intraradices*, *G. mosseae* or *G. dussii* granular inoculum. The AMF granules were poured into the planting holes at a depth of 10 cm in each pot

and the tubers (one per pot) were then planted with their developing roots in contact with the mycorrhizal granules. Soil was added to the pots to a level at which sprouts were just visible. Plants were watered as needed and each week, each plant received 500 ml of the nutrient solution described earlier (12 applications in total).

### **Trial site and design**

The experimental units (potted plants) were placed outdoors in the experimental garden of the Department of Plant Science (The National University of Ireland, Cork) in a factorial 5 x 2 x 2 randomised block design comprising four AMF treatments and an untreated control, two nematicide treatments (presence, absence) and two *Globodera pallida* treatments (presence, absence). Each of the 20 treatments was replicated six times for each of the three sampling dates (2, 4 and 12 wk after planting), making a total of 360 pots for the entire experiment.

### **Sampling**

Six replicate pots from each of the 20 treatments (5 AMF treatments x 2 nematicide x 2 PCN treatments) were sampled at each of the three harvest dates (2, 4 and 12 wk after planting). A number of parameters were assessed, including percentage hatch of viable eggs at 2 and 4 wk, root and shoot dry weight at 2 and 4 wk, percentage root length colonisation by AMF at 4 wk and final nematode population ( $P_f$ ), multiplication ( $P_f/P_i$ ) and yield at 12 wk.

## Assessments

### Percentage hatch

At 2 and 4 wk after planting, pots containing sachets with PCN cysts (six replicate pots from each +PCN treatment at each harvest date) were emptied, the sachets were recovered and the percentage hatch of viable eggs was determined using the methodology of Byrne *et al.* (1998).

### Root and shoot dry weight

Potato plants from six replicate pots from all 20 treatments were removed at 2 and at 4 wk after planting. After the root systems were rinsed thoroughly with tap water to remove soil particles from the root system, each plant was divided into roots and shoots. Root and shoot dry weight of each plant was determined by oven-drying of the respective tissues for 48 h at 60°C.

### Root length colonisation by AMF

Assessment of mycorrhizal colonisation was conducted on root samples of AMF-inoculated plants harvested 4 wk after planting. Root samples were taken from six replicate pots in each treatment. The percentage AMF root colonisation was determined using the grid line intersect method of Giovannetti & Mosse (1980).

### Nematode multiplication

The number of viable eggs g<sup>-1</sup> soil at planting (initial nematode population density,  $P_i$ ) and at 12 wk after planting (final nematode population density,  $P_f$ ) were used to calculate the PCN multiplication, which was expressed as  $P_f/P_i$ . Since the soil used was free of PCN, multiplication was estimated only from those pots in which

PCN inoculum (60 cysts) had been added at planting. The  $P_i$  was determined using the original number of cysts per pot (60), the mean number of eggs per cyst and the percentage viability of eggs. To determine the  $P_f$  in each replicate pot after 12 wk, the soil content of each pot (c. 8.2 kg) was thoroughly mixed in a tray (1.2 x 0.6 x 0.2 m) and sufficient number of soil subsamples (approximately 20) were taken at random to give a bulk soil sample of c. 1 kg, which was then bagged and dried at 30°C for 5 days. Following this, the cysts from a 200 g subsample of dried soil were recovered using a Wye Washer elutriator (Winfield *et al.*, 1987) and their number counted. The  $P_f$  in each sample was then calculated as for the  $P_i$ , using the number of cysts per pot, the mean number of eggs per cyst and the percentage viability of eggs. Egg numbers and percentage viability were determined using the same methodology described above.

### **Tuber yield**

Tubers from six replicate plants in each treatment were harvested, counted and weighed at 12 wk after planting, the mean values were determined and the result was expressed as total fresh tuber weight per plant.

### **Data analysis**

Statistical analysis on the data was conducted by parametric interaction analysis of variance (ANOVA; Table 2: two-way, Tables 1 and 3 and percentage root length colonisation: three-way, Figs 1 and 2: four-way) using the Datadesk Release 5.0 programme for Macintosh (Data Description, Inc., Ithaca, New York). Individual one-way ANOVA was also conducted in all data sets in order to highlight the effect of AMF as a single factor on the various parameters assessed. Multiple comparison

tests between more than two treatments were conducted using Fisher's Protected Least Significant Difference (LSD). Total eggs cyst<sup>-1</sup>, viable eggs cyst<sup>-1</sup>, viable eggs g<sup>-1</sup> soil, cysts plant<sup>-1</sup> and tuber yield data were square root transformed prior to parametric analysis in order to obtain variance homogeneity.

## Results

### Root length colonisation by AMF

Microscopical observations conducted on root samples from AMF-inoculated plants at 4 wk after planting revealed that all AMF isolates colonised successfully the potato root system of plants in all treatments. While no differences in the percentage root length colonisation were found between nematicide treated and untreated plants, i.e. there was no significant nematicide main effect, the main effect of AMF was highly significant ( $P < 0.001$ , SED = 1.721). The LSD tests indicated that the grand mean percentage root colonisation by Vaminoc (51.5%) was higher than all other AMF treatments and that colonisation by *G. intraradices* (44.3%) and *G. mosseae* (46.8%) while it did not differ between the two it was higher than that exhibited by *G. dussii* (28.0%). There was also a significant main effect of PCN on percentage root colonisation ( $P < 0.01$ ); PCN inoculation of potato plants reduced significantly the percentage mycorrhizal root colonisation (with PCN: 40.6%; without PCN: 44.7%). There was no convincing evidence of a three-way interaction between AMF, PCN and nematicide for root length colonisation nor were any of the three two-way interactions significant.

The one-way ANOVA of percentage root length colonisation by AMF (after pooling plus and minus nematicide data) detected a highly significant  $F$ -ratio for AMF treatments in both the presence and absence of PCN [with PCN:  $F(3,47) =$

33.75,  $P < 0.001$ , SED = 2.414; without PCN:  $F(3,47) = 40.69$ ,  $P < 0.001$ , SED = 2.354].

### Percentage hatch

The one-way ANOVA of percentage hatch of viable eggs for AMF treatments (Table 1) was significant at 2 wk after planting in the presence of nematicide [ $F(4, 25) = 3.47$ ,  $P < 0.05$ , SED = 4.400]. Multiple comparison tests between treatments revealed that inoculation of potato roots with Vaminoc and *G. intraradices* caused a significant stimulation of *G. pallida* hatch at the first 2 wk only in the presence of nematicide. By this time, in the absence of nematicide, inoculation of potato plants with Vaminoc and with its individual components did not increase significantly the in-soil hatch of *G. pallida* [ $F(4, 25) = 0.46$ ].

After 4 wk, under nematicide-treated plants, the presence of mycorrhizal fungi on the potato roots did not affect significantly the hatch of *G. pallida* [ $F(4, 25) = 1.57$ ]. The one-way ANOVA at wk 4 in plants not treated with nematicide, revealed a significant effect of AMF on the in-soil hatch of *G. pallida* [ $F(4, 25) = 5.47$ ,  $P < 0.01$ , SED = 3.674]; subsequent comparisons between treatments showed that the mean percentage hatch of *G. pallida* from *G. intraradices*-inoculated plants (82.3%) was significantly greater not only than that from non-mycorrhizal plants but also than the hatch from the other AMF-inoculated plants (Table 1).

In the absence of AMF, nematicide application did not produce any significant effect on the in-soil hatch of *G. pallida* at either 2 or 4 wk (Table 1).

**Table 1. Effect of inoculation of potato cv. Golden Wonder with four inocula of arbuscular mycorrhizal fungi (AMF) (NM: non-mycorrhizal, M1: Vaminoc, M2: *Glomus intraradices*, M3: *Glomus mosseae* and M4: *Glomus dussii*) on the in-soil hatch of *Globodera pallida* at 2 and 4 wk after planting in the presence and absence of nematicide (NMT). Any two samples sharing a common letter in the same row were not significantly different at the  $P = 0.05$  level.**

Date	NMT	In-soil hatch of <i>G. pallida</i> (%)					<i>P</i>
		NM	M1	M2	M3	M4	
2 wk	+	54.1a	65.2b	66.8b	54.9a	59.2ab	<0.05
	-	59.9	63.0	62.4	64.9	66.8	>0.05
4 wk	+	65.5	72.1	73.8	74.5	73.6	>0.05
	-	66.7a	68.7a	82.3b	70.8a	71.1a	<0.01
s.e. of difference (SED) between any two means = 4.450							
Source of variation		df	Mean square	<i>F</i> -ratio	<i>Probability</i>		
Date (DAT)		1	3121.40	52.54	<0.001		
Nematicide (NMT)		1	87.18	1.47	>0.05		
AMF		4	295.40	4.97	<0.01		
DAT x NMT		1	81.02	1.36	>0.05		
DAT x AMF		4	52.00	0.88	>0.05		
NMT x AMF		4	39.50	0.66	>0.05		
DAT x NMT x AMF		4	158.44	2.67	<0.05		
Error		100	59.41	-	-		

+, nematicide added; -, nematicide not added

The interaction ANOVA of percentage hatch (Table 1) showed that from 2 to 4 wk there was a significant increase (from 61.7% to 71.9%;  $P < 0.001$ ) in the in-soil hatch of *G. pallida* (significant date main effect). Analysis of the significant AMF main effect ( $P < 0.01$ , SED = 2.225) demonstrated that the hatch of *G. pallida* was significantly higher from AMF-inoculated plants as compared to non-mycorrhizal plants (mean of 61.6%); this effect was observed with all four AMF isolates, while within AMF the only significant difference was between *G. mosseae* (mean of

66.3%) and *G. intraradices* (mean of 71.3%). Nematicide application had no direct effect on the hatch of *G. pallida* (no significant nematicide main effect); the only effect of nematicide was in the date x nematicide x AMF interaction. While the three-way interaction between date, nematicide and AMF on hatch was significant ( $P < 0.05$ , SED = 4.450), all three two-way interactions were not significant (Table 1). When the statistical analysis of the results was repeated by pooling the plus/minus nematicide data, it was shown that mycorrhizal inoculation had increased the hatch of *G. pallida* only after 4 wk [ $F(4, 55) = 4.69$ ,  $P < 0.01$ , SED = 1.785].

### Root and shoot dry weight

Because a 2 g fresh root sample had been removed from all AMF-inoculated plants to evaluate the percentage mycorrhizal root colonisation at wk 4 and in order to achieve similar standards in all plants in the estimation of root dry weight, the recorded root dry weight values from all mycorrhizal plants at wk 4 were corrected by adding 0.03 g in each sample; 0.03 g was found to be the equivalent dry weight of the 2 g fresh root sample and it was the mean of 10 replications (SE = 0.003). Root dry weight results are described in Fig. 1, while those from shoot dry weight are shown in Fig. 2.

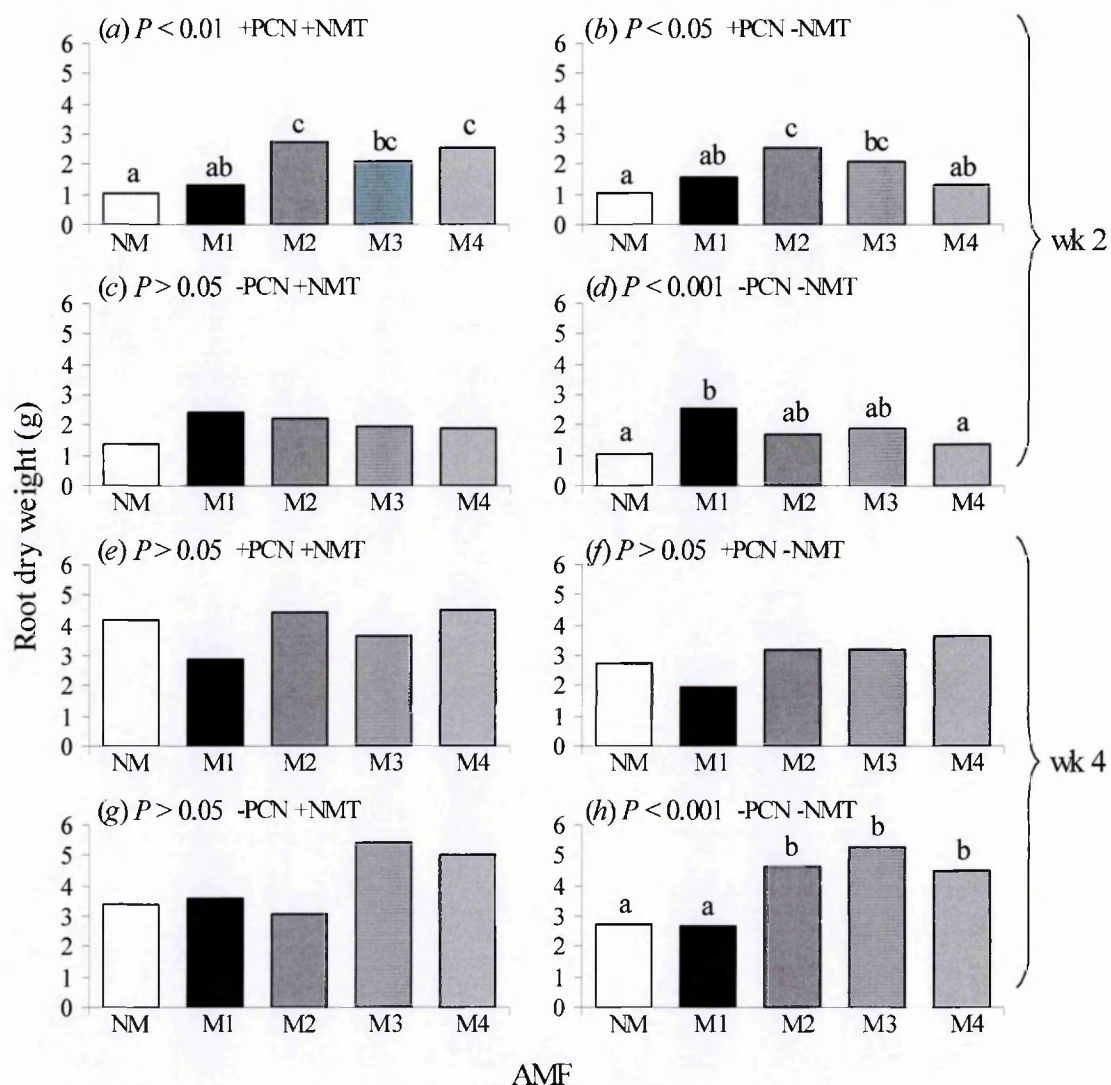
At wk 2, inoculation of potato roots with Vaminoc produced a significant stimulatory effect on root growth only in the minus PCN/minus nematicide treatment [ $F(4, 25) = 7.92$ ,  $P < 0.001$ , SED = 0.281]. The lack of a similar effect of Vaminoc on root dry weight in the rest of the treatments indicated that PCN inoculation and nematicide application separately or in combination eliminated the significant effect of Vaminoc on roots. By this time, from the individual components of the Vaminoc mixture, same effects were produced by all components in the presence of PCN and



nematicide [ $F(4, 25) = 4.94$ ,  $P < 0.01$ ,  $SED = 0.475$ ] and by two of the components (*G. intraradices* and *G. mosseae*) in the presence of PCN only [ $F(4, 25) = 3.28$ ,  $P < 0.05$ ,  $SED = 0.480$ ]. In the treatment that included nematicide but no PCN, no significant effect of AMF on roots was detected, demonstrating that in the absence of PCN, nematicide application eliminated the significant effect of the individual AMF isolates on root dry weight.

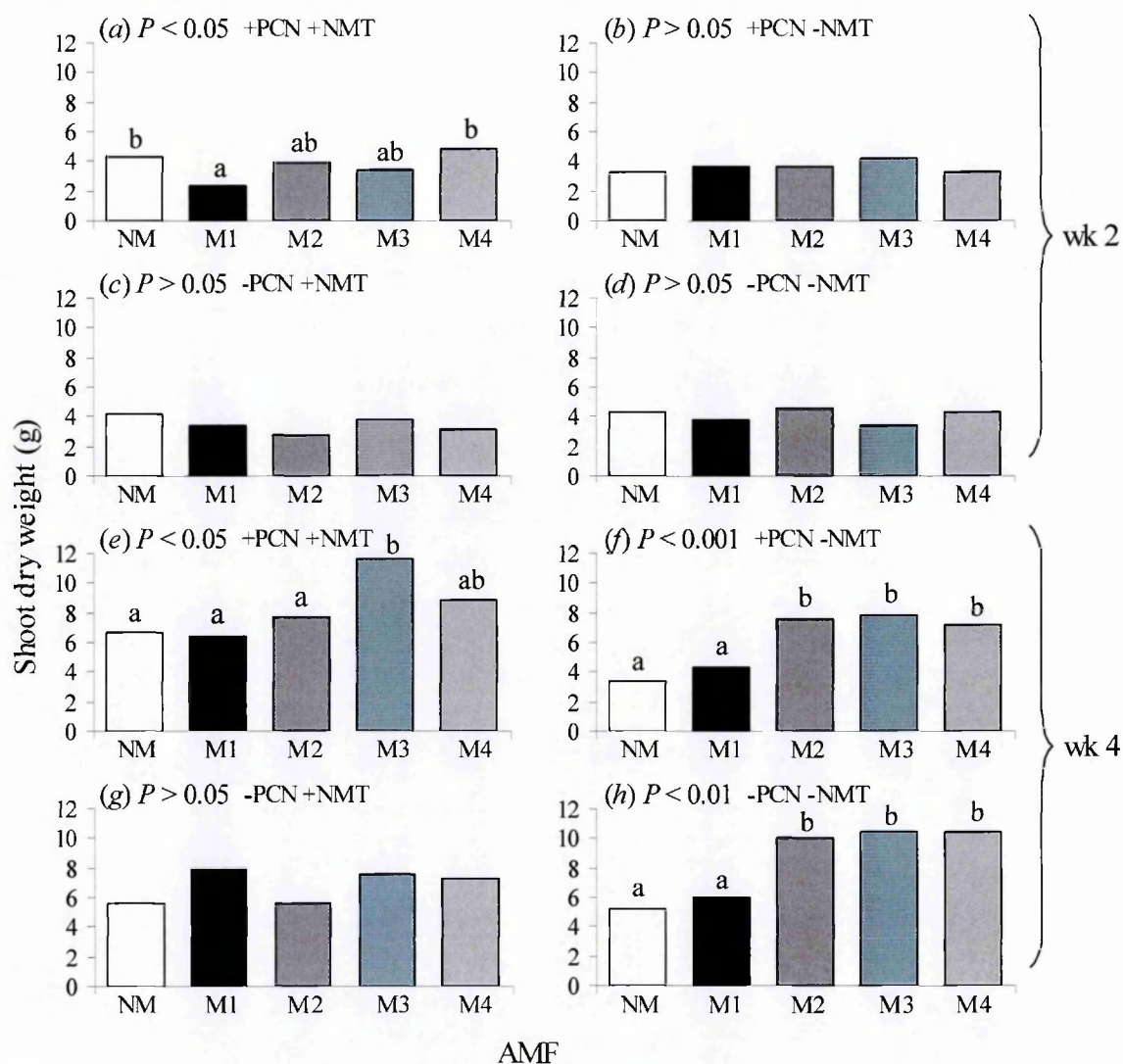
After 4 wk, there was less evidence of mycorrhizal stimulation of root growth; only in the absence of PCN and nematicide did AMF-inoculated plants exhibit significantly greater root dry weights than the non-mycorrhizal plants [ $F(4, 25) = 7.12$ ,  $P < 0.001$ ,  $SED = 0.626$ ]. This result was exhibited by the three individual *Glomus* species but not with the Vaminoc mixture. The absence of a significant effect of the individual AMF isolates on roots in treatments involving PCN and nematicide (both or in isolation) demonstrated that PCN inoculation and nematicide application eliminated the effect of AMF on root dry weight. Vaminoc-inoculation of potato plants did not produce any effect on roots after 4 wk in any of the treatments.

In contrast with root dry weight, in shoot dry weight, the significant effect of mycorrhization was restricted to wk 4 and was totally absent at wk 2. At 4 wk after planting, in the absence of PCN and nematicide, shoot dry weights of plants inoculated with the individual AMF isolates were significantly greater than of non-mycorrhizal plants and of plants inoculated with Vaminoc [ $F(4, 25) = 5.09$ ,  $P < 0.01$ ,  $SED = 1.616$ ]; similar trends for shoot dry weight were obtained for mycorrhizal and non-mycorrhizal plants grown in the presence of PCN only, i.e. without added nematicide [ $F(4, 25) = 8.16$ ,  $P < 0.001$ ,  $SED = 1.019$ ]. The significant effect of the individual AMF isolates on shoot dry weight was largely eliminated when nematicide was added to the pots; in the presence of nematicide, only



Main effects and interactions on root dry weight			
Source of variation	F-ratio (P)	Source of variation	F-ratio (P)
AMF <sup>4</sup> df	9.72 (<0.001)	DAT x NMT <sup>1</sup> df	1.23 (>0.05)
DAT <sup>1</sup> df	203.93 (<0.001)	PCN x NMT <sup>1</sup> df	2.44 (>0.05)
PCN <sup>1</sup> df	4.82 (<0.05)	AMF x DAT x PCN <sup>4</sup> df	3.19 (<0.05)
NMT <sup>1</sup> df	9.04 (<0.01)	AMF x DAT x NMT <sup>4</sup> df	1.36 (>0.05)
AMF x DAT <sup>4</sup> df	5.91 (<0.001)	AMF x PCN x NMT <sup>4</sup> df	0.71 (>0.05)
AMF x PCN <sup>4</sup> df	3.51 (<0.01)	DAT x PCN x NMT <sup>1</sup> df	2.66 (>0.05)
AMF x NMT <sup>4</sup> df	0.91 (>0.05)	AMF x DAT x PCN x NMT <sup>4</sup> df	1.35 (>0.05)
DAT x PCN <sup>1</sup> df	4.40 (<0.05)	Error <sup>200</sup> df	-

Fig. 1



Main effects and interactions on shoot dry weight

Source of variation	F-ratio (P)	Source of variation	F-ratio (P)
AMF <sup>4 df</sup>	8.97 (<0.001)	DAT x NMT <sup>1 df</sup>	1.03 (>0.05)
DAT <sup>1 df</sup>	211.19 (<0.001)	PCN x NMT <sup>1 df</sup>	20.86 (<0.001)
PCN <sup>1 df</sup>	1.00 (>0.05)	AMF x DAT x PCN <sup>4 df</sup>	0.41 (>0.05)
NMT <sup>1 df</sup>	0.03 (>0.05)	AMF x DAT x NMT <sup>4 df</sup>	2.28 (>0.05)
AMF x DAT <sup>4 df</sup>	9.43 (<0.001)	AMF x PCN x NMT <sup>4 df</sup>	2.18 (>0.05)
AMF x PCN <sup>4 df</sup>	1.07 (>0.05)	DAT x PCN x NMT <sup>1 df</sup>	9.13 (<0.01)
AMF x NMT <sup>4 df</sup>	3.06 (<0.05)	AMF x DAT x PCN x NMT <sup>4 df</sup>	1.48 (>0.05)
DAT x PCN <sup>1 df</sup>	0.55 (>0.05)	Error <sup>200 df</sup>	-

Fig. 2

**Fig. 1.** Root dry weight (g) of non-mycorrhizal plants (NM) and of plants inoculated with the arbuscular mycorrhizal fungi (AMF) *Vaminoc* (M1), *Glomus intraradices* (M2), *Glomus mosseae* (M3) and *Glomus dussii* (M4) at 2 and 4 wk after planting [(a) + PCN, + nematicide, 2 wk, (b) + PCN, - nematicide, 2 wk, (c) - PCN, + nematicide, 2 wk, (d) - PCN, - nematicide, 2 wk, (e) + PCN, + nematicide, 4 wk, (f) + PCN, - nematicide, 4 wk, (g) - PCN, + nematicide, 4 wk, (h) - PCN, - nematicide, 4 wk]. Any two columns sharing a common letter in subfigures (a), (b), (d) and (h) were not significantly different at the  $P = 0.05$  level (DAT = date; NMT = nematicide).

*G. mosseae*-inoculated PCN-infested plants exhibited greater shoot dry weights than the non-mycorrhizal plants [ $F(4, 25) = 3.81, P < 0.05, SED = 1.554$ ].

Application of aldicarb nematicide had a significant ( $P < 0.05$ ) stimulatory effect on shoot dry weight of the non-mycorrhizal PCN-infested plants after 4 wk (but not after 2 wk). On the other hand, non-mycorrhizal PCN-infested plants treated with aldicarb exhibited similar root dry weights with their untreated counterparts at both harvest dates.

All four factors exhibited a significant main effect on root dry weight (AMF,  $P < 0.001$ ; date,  $P < 0.001$ ; PCN,  $P < 0.05$ ; nematicide,  $P < 0.01$ ), with the means for non-mycorrhizal plants, *Vaminoc*-, *G. intraradices*-, *G. mosseae*- and *G. dussii*-inoculated plants being 2.19, 2.37, 3.07, 3.19 and 3.10 g (LSD = 0.418), respectively, for 2 wk and 4 wk 1.83 and 3.74 g, respectively, for PCN-infested and non-PCN-infested plants 2.64 and 2.93 g, respectively, and for nematicide treated and untreated plants 2.99 and 2.58 g, respectively. There were three significant 2-way interactions. These existed between AMF and date ( $P < 0.001$ ), between AMF and PCN ( $P < 0.01$ ) and between date and PCN ( $P < 0.05$ ). The significant AMF x date interaction on root dry weight indicated that the effect of AMF on roots depended greatly on the plant age; after 2 wk all mycorrhizal treatments exhibited increased root dry weight compared to plants which did not receive mycorrhizal treatment, whereas after 4 wk similar effects were produced only by *G. mosseae* and *G. dussii*. Closer examination

**Fig. 2.** Shoot dry weight (g) of non-mycorrhizal plants (NM) and of plants inoculated with the arbuscular mycorrhizal fungi (AMF) Vaminoc (M1), *Glomus intraradices* (M2), *Glomus mosseae* (M3) and *Glomus dussii* (M4) at 2 and 4 wk after planting [(a) + PCN, + nematicide, 2 wk, (b) + PCN, - nematicide, 2 wk, (c) - PCN, + nematicide, 2 wk, (d) - PCN, - nematicide, 2 wk, (e) + PCN, + nematicide, 4 wk, (f) + PCN, - nematicide, 4 wk, (g) - PCN, + nematicide, 4 wk, (h) - PCN, - nematicide, 4 wk]. Any two columns sharing a common letter in subfigures (a), (e), (f) and (h) were not significantly different at the  $P = 0.05$  level (DAT = date; NMT = nematicide).

of the AMF x PCN interaction on root dry weight revealed that PCN inoculation reduced the root dry weights of the Vaminoc- and *G. intraradices*-inoculated plants only, while mycorrhizal inoculation increased the root dry weights of plants inoculated with the three individual AMF isolates in the presence of PCN and of plants inoculated with all AMF isolates in the absence of PCN. While after 2 wk PCN inoculation had not affected root dry weight, by wk 4, PCN-infested plants were exhibiting significantly smaller root systems than the non-PCN-infested plants. The only significant three-way interaction for root dry weight existed between AMF, date and PCN ( $P < 0.05$ ). There was no significant four-way interaction between AMF, date, PCN and nematicide when comparing root dry weight (Fig. 1).

There were highly significant differences in shoot dry weight among AMF ( $P < 0.001$ ), with the means for non-mycorrhizal plants, Vaminoc-, *G. intraradices*-, *G. mosseae*- and *G. dussii*-inoculated plants being 4.63, 4.72, 5.73, 6.51 and 6.16 g (LSD = 0.789), respectively, and among date ( $P < 0.001$ ), with mean values of 3.72 and 7.34 g at wk 2 and at wk 4, respectively. No significant main effect of PCN on shoot dry weight was detected nor was the nematicide main effect significant at all. There was a highly significant AMF x date interaction on shoot dry weight ( $P < 0.001$ ); while after 2 wk none of the four AMF had affected the shoot dry weight of plants, after 4 wk, inoculation with the three individual AMF isolates resulted in significant increases in shoot dry weight. Interaction ANOVA also detected a highly

significant PCN x nematicide interaction ( $P < 0.001$ ); interestingly PCN and nematicide when added separately to the pots, they both had a significant detrimental effect on the shoot dry weight of plants, as compared to when they were added together to the pots or to when neither of them was added. Nematicide also interacted significantly with AMF on shoot dry weight ( $P < 0.05$ ). In particular, nematicide application stimulated the shoot growth of non-mycorrhizal plants, but had either a detrimental (*G. intraradices*) effect or no absolute effect (rest of the AMF) on shoots of mycorrhizal plants. Furthermore, the effect (significant increase, compared to non-mycorrhizal plants) of two of the three individual Vaminoc components (*G. intraradices* and *G. dussii*) on shoot dry weight was eliminated when plants were treated with nematicide. However, the three-way interactions on shoot dry weight were in absolute contrast with those on root dry weight. While there was no significant AMF x date x PCN interaction, all the other three-way interactions were significant. Similarly to root dry weight, the four-way interaction ANOVA did not detect any significant interaction between AMF, date, PCN and nematicide for shoot dry weight (Fig. 2).

When the different treatments were compared in terms of total dry biomass produced per plant (data not shown), it was revealed that mycorrhization of potato roots had affected plant growth only after 4 wk and had no effect at all after 2 wk. The effect of AMF on enhancing the potato plant growth was totally restricted to the individual AMF isolates and it was consistent (i.e. exhibited by all the individual AMF isolates) and highly significant ( $P < 0.001$ ) only in the absence of nematicide (with/without PCN). As observed with shoot dry weight, application of nematicide limited the positive effect of AMF on plant growth; in the presence of PCN, only *G. mosseae*-inoculated plants exhibited significantly ( $P < 0.05$ ) increased total dry

biomass compared to non-mycorrhizal controls, while in the absence of PCN no differences were detected among the treatments. The interaction ANOVA results of total dry biomass were similar to that of shoot dry weight, with the only noticeable difference being that of the PCN main effect (total dry biomass:  $P = 0.014$ ; shoot dry weight:  $P = 0.319$ ), apparently because of the significant PCN main effect on root dry weight ( $P = 0.029$ ).

### Nematode multiplication

The mean number of eggs cyst<sup>-1</sup> prior to the establishment of the trial was 789 ( $\pm 30.6$ ) with an average viability of 88.0% ( $\pm 1.91$ ). The initial PCN inoculum was 60 cysts pot<sup>-1</sup> and the initial nematode population density ( $P_i$ ) was 5 eggs g<sup>-1</sup> soil ( $\pm 0.19$ ). The results on cyst production and *G. pallida* population increase (after 12 wk) are summarised in Table 2.

Inoculation of potato roots with the four AMF isolates had no effect on the number of total eggs cyst<sup>-1</sup> and viable eggs cyst<sup>-1</sup> in both the presence and absence of nematicide at 12 wk after planting and there was no significant nematicide main effect and hence, no AMF x nematicide interaction.

In the absence of nematicide, significantly higher numbers of cysts plant<sup>-1</sup> were found on non-mycorrhizal than in mycorrhizal plants from all four treatments, but no differences were detected among the four types of AMF plants [ $F(4, 25) = 13.27$ ,  $P < 0.001$ ]. When plants were treated with nematicide, almost similar trends were revealed, with all AMF-inoculated plants producing significantly lower numbers of cysts plant<sup>-1</sup>, compared to non-mycorrhizal plants [ $F(4, 25) = 7.30$ ,  $P < 0.001$ ]. This time however, differences among AMF treatments were apparent, with *G. dussii*-inoculated plants producing significantly more cysts plant<sup>-1</sup> than the Vaminoc- and

*G. intraradices*-inoculated plants. Nematicide did not interact significantly with AMF on cyst numbers plant<sup>-1</sup>, but it had a significant main effect ( $P < 0.001$ ). The reduction on cyst numbers per plant as a result of nematicidal application was high and ranged from 56% in *G. dussii*-inoculated plants to 122% in Vaminoc-inoculated plants.

**Table 2. Effect of applying both arbuscular mycorrhizal fungi (AMF) and aldicarb nematicide (NMT) at planting on the population change of *Globodera pallida* (NM: non-mycorrhizal, M1: Vaminoc, M2: *Glomus intraradices*, M3: *Glomus mosseae* and M4: *Glomus dussii*). Any two samples sharing a common letter in the same row were not significantly different at the  $P = 0.05$  level.**

Character	NMT	AMF treatment <sup>a</sup>					<i>P</i>
		NM	M1	M2	M3	M4	
cysts plant <sup>-1</sup>	+	570 (23.63)c	247 (15.45)a	268 (16.21)a	288 (16.79)ab	405 (19.91)b	<0.001
	-	1160 (33.91)b	549 (23.25)a	494 (22.02)a	576 (23.85)a	631 (24.98)a	
viable eggs g <sup>-1</sup> soil	+	37 (6.03)b	17 (4.00)a	19 (4.33)a	21 (4.52)a	27 (5.08)ab	<0.01
	-	90 (9.35)b	39 (6.15)a	33 (5.56)a	39 (6.13)a	41 (6.40)a	
$P_f/P_i$	+	7.30b	3.37a	3.81a	4.14a	5.30ab	<0.01
	-	17.73b	7.73a	6.48a	7.64a	8.13a	<0.001
Analysis of variance of $P_f/P_i$ ratio							
Source of variation	df	Mean square	<i>F</i> -ratio	<i>Probability</i>			
NMT	1	339.01	44.99	< 0.001			
AMF	4	111.46	14.79	< 0.001			
NMT x AMF	4	31.50	4.18	< 0.01			
Error	50	7.54	-	-			

<sup>a</sup> Square-root transformed data (to normalise) in parentheses

+, nematicide added; -, nematicide not added



The final population size of *G. pallida* eggs  $\text{g}^{-1}$  soil on mycorrhizal plants was significantly lower than that on non-mycorrhizal plants in both the presence and absence of nematicide. As a result, the multiplication ( $P_f/P_i$ ) of *G. pallida* was also significantly lower on mycorrhizal than on non-mycorrhizal plants. In the absence of nematicide, the mean  $P_f/P_i$  ratio on non-mycorrhizal plants was  $17.7 (\pm 2.34)$ ; mycorrhizal inoculation of potato roots with all AMF reduced this ratio by over 50% [ $F(4, 25) = 10.89, P < 0.001, \text{SED} = 1.979$ ]. The one-way ANOVA for  $P_f/P_i$  in the plus nematicide treatment detected a significant  $F$ -ratio for AMF treatments [ $F(4, 25) = 4.51, P < 0.01, \text{SED} = 1.051$ ]. When pots were treated with nematicide, the multiplication of *G. pallida* on non-mycorrhizal plants ( $7.30 \pm 0.76$ ) was reduced significantly ( $P < 0.001$ ), but it was still significantly higher ( $P < 0.05$ ) than the  $P_f/P_i$  on plants colonised by *Vaminoc*, *G. intraradices* and *G. mosseae*.

The two-way interaction ANOVA of  $P_f/P_i$  (Table 2) revealed the presence of a significant AMF and nematicide main effect ( $P < 0.001$  for both) and of a significant AMF x nematicide interaction ( $P < 0.01$ ). When the mean values of each factor (AMF, nematicide) were averaged over the levels of the other factor, it was revealed that both mycorrhizal inoculation of potato roots and application of nematicide had resulted in reducing the *G. pallida* population increase by about 50%. The AMF x nematicide interaction on *G. pallida* multiplication was largely the effect of the *G. dussii* inoculum. Closer examination of the interaction results showed that the positive effect of AMF inoculation on  $P_f/P_i$  (i.e. smaller population increase compared to non-mycorrhizal plants) was greater in the absence than in the presence of nematicide, due to the lack of a significant effect of *G. dussii* on  $P_f/P_i$  in the plus nematicide treatment. Analysis of the interaction showed also that the positive effect of aldicarb application was greater for non-mycorrhizal plants ( $P < 0.001$ ), *Vaminoc*-

( $P < 0.01$ ) and *G. mosseae*-inoculated plants ( $P < 0.05$ ), than for *G. intraradices*- ( $P = 0.09$ ) and *G. dussii*-inoculated plants ( $P = 0.08$ ).

## Yield

Total fresh tuber yield per plant was assessed at 12 wk after planting and the results are described in Table 3. In the absence of PCN and nematicide, plants that had been inoculated with the three single *Glomus* isolates gave higher yields than the non-mycorrhizal plants. The *F*-ratio for AMF treatments ( $df = 4, 25$ ) was 2.11 ( $P < 0.05$ ). Nevertheless, when plants were grown in the presence of PCN and nematicide (separately or in combination) tuber yields of AMF-inoculated plants did not differ significantly from the yields of non-mycorrhizal plants.

The AMF main effect on tuber yield was highly significant ( $P < 0.001$ ; *G. intraradices*, *G. mosseae*, *G. dussii* > non-mycorrhizal) and so was the nematicide main effect ( $P < 0.001$ ; treated plants > untreated). In contrast, the PCN main effect was not significant. The only significant two-way interaction was between PCN and nematicide ( $P < 0.01$ ); PCN-infested plants not treated with nematicide gave the lowest tuber yields. The three factors (AMF, PCN, nematicide) did not produce a significant interaction on tuber yield.

## Discussion

Since both group of organisms (AMF and PCN) colonise the same area of root tissue, there is an increased probability that they will interact biologically. There have been several studies examining the interactions between plant-parasitic nematodes (other than PCN) and AMF on a wide range of plants (e.g. Hussey & Roncadori, 1982; Ingham, 1988). In the current study, AMF and *G. pallida* were

**Table 3. Total fresh tuber weight (g) per plant at 12 wk after planting (NMT: nematicide, NM: non-mycorrhizal plants, M1: Vaminoc-, M2: *Glomus intraradices*-, M3: *Glomus mosseae*- and M4: *Glomus dussii*-inoculated plants). Any two samples sharing a common letter in the same row were not significantly different at the  $P = 0.05$  level.**

PCN/NMT	AMF treatment <sup>a</sup>					<i>P</i>
	NM	M1	M2	M3	M4	
+/+	95.70 (9.689)	98.03 (9.866)	99.00 (9.884)	129.57 (11.301)	119.47 (10.906)	>0.05
+/-	58.45 (7.568)a	71.12 (8.391)ab	85.88 (9.197)bc	100.40 (9.898)c	86.30 (9.186)bc	>0.05
-/+	101.12 (10.030)	100.08 (9.893)	96.85 (9.789)	121.13 (11.003)	92.65 (9.530)	>0.05
-/-	73.07 (8.450)a	92.52 (9.567)ab	125.20 (11.121)c	109.10 (10.361)bc	104.50 (10.199)bc	<0.05
Source of variation		df	Mean square	<i>F</i> -ratio	<i>Probability</i>	
AMF		4	9.93	6.43	<0.001	
PCN		1	4.94	3.20	>0.05	
NMT		1	18.98	12.30	<0.001	
AMF x PCN		4	1.18	0.77	>0.05	
AMF x NMT		4	3.75	2.43	>0.05	
PCN x NMT		1	14.10	9.13	<0.01	
AMF x PCN x NMT		4	0.96	0.62	>0.05	
Error		100	1.54	-	-	

<sup>a</sup> Square-root transformed data (to normalise) in parentheses

+, PCN and/or nematicide added; -, PCN and/or nematicide not added

mutually inhibitory, each reducing the population of the other. However, because nematode-AMF interactions are highly specific and vary with the particular host-AMF-nematode combination (Ingham, 1988), generalisations on the effects of AMF on plant growth and nematode development are difficult. Nematode-AMF interactions are also very important in agricultural ecosystems, where suppression of the nematode population by AMF is highly desirable for enhanced agronomic yield and decreased disease incidence of the crop. Smith (1988) reported that concomitant infections of plant-parasitic nematodes and AMF are the rule rather than the exception in roots of agricultural crops, due to the ubiquitous occurrence of AMF in most agricultural soils and their complete dependence on host roots.

The significant increase in the in-soil hatch of *G. pallida* at early stages of plant growth, as expressed by the significant AMF main effect, following mycorrhizal inoculation of the potato root confirmed that AMF (mixed or pure isolates) have a potential to interfere in the *G. pallida* hatching mechanism. The current results supported previous reports from *in vitro* and in-sand studies conducted on the same cultivar (Golden Wonder), that the early hatch of *G. pallida* is stimulated by the activity of mycorrhizal fungi (Ryan *et al.*, 2000; Ryan *et al.*, 2003; Deliopoulos *et al.*, 2004). However this time, in addition to Vaminoc, the effects of three single AMF isolates (components of Vaminoc) on the hatch of *G. pallida* were tested, under conditions resembling the situation in the field. The results showed that the component isolates did not exhibit better characteristics compared to Vaminoc with regard to the effect on *G. pallida* hatch; clearly the effects of the three single AMF isolates on hatch were not additive towards Vaminoc. From the single AMF isolates the most hatch-active appeared to be *G. intraradices*. The significant date main effect combined with the absence of a significant AMF x date interaction

suggests that the increase in the hatch of *G. pallida* with time (from 2 to 4 wk) did not depend on the AMF treatment. Under non-mycorrhizal plants, there was a significant increase in hatch from 2 to 4 wk, indicating that the early in-soil hatch of *G. pallida* was delayed; late in-soil hatch of *G. pallida* has also been reported by Whitehead (1992). Interestingly, the percentage of *G. pallida* hatched J<sub>2</sub>s after 4 wk under non-mycorrhizal plants was similar to that exhibited under mycorrhizal plants after just 2 wk, demonstrating that AMF inoculation of potato plants accelerated the in-soil hatch of *G. pallida*, eliminating thus the recorded delay in *G. pallida* hatch under non-mycorrhizal plants. Although mycorrhization resulted in increasing the hatch of *G. pallida* (as compared to the hatch from minus AMF plants), the observation that hatch increased significantly from 2 to 4 wk even under mycorrhizal plants, suggests that it is probably unlikely that mycorrhizal inoculation of potato plants would have such a large effect in the hatch of *G. pallida* so that within 2 wk from planting the majority of J<sub>2</sub>s would have emerged. This mycorrhizal-induced stimulation of *G. pallida* hatch was not associated, in the majority of the cases, with a parallel increase in the root dry weights of plus AMF, as compared to minus AMF plants. For example, after 4 wk, in the presence of PCN (plus/minus nematicide) root dry weight was similar from plus AMF and minus AMF plants, but the percentage hatch of *G. pallida* was higher under plus AMF plants, compared to minus AMF plants (as shown after pooling the plus/minus nematicide data on percentage hatch). Recent work has shown that the stimulation of the hatch of *G. pallida* by mycorrhizal fungi is due to the altered hatching chemical production by the potato plant and/or due to the production of novel mycorrhizal HFs or HSs. Ryan & Jones (2003b) found that PRL from Vaminoc-inoculated plants contained increased amounts of HFs and HSs (relative to the PRL from non-mycorrhizal plants) with activity towards *G.*

*pallida*; Deliopoulos *et al.* (2004) reported that PRL from Vaminoc-, *G. intraradices*- and *G. mosseae*-inoculated plants produced a number of *G. pallida*-active HF's which were not detected in the PRL from non-mycorrhizal plants.

In contrast with AMF, aldicarb application at the recommended field rate (25 mg kg<sup>-1</sup> soil or 200 mg pot<sup>-1</sup>) did not appear to have any effect on the hatch of *G. pallida* at either observation date, i.e. at 2 and 4 wk after planting. Hague & Pain (1973) reported that the principal mode of action of aldicarb (nematostasis) has been shown to be against the soil phase of the nematode life cycle, i.e. after nematodes have hatched rather than when the J<sub>2</sub> is still within the encysted egg; this possibly explains why aldicarb failed to cause any inhibition (or stimulation) in the hatch of *G. pallida*. Extensive studies have been conducted in the past on the effects of granular oximecarbamate nematicides (oxamyl, aldicarb) on PCN hatch (Hague & Pain, 1970, 1973; Evans & Wright, 1982), but the majority of them were undertaken *in vitro* and focused more on *G. rostochiensis* rather than *G. pallida*; Osborne (1973) reported that aldicarb concentrations above 1 ppm caused a long-term inhibition of hatch in *G. rostochiensis*.

Similarly to the in-soil hatch of *G. pallida*, the level of mycorrhizal infection in the potato root system was also unaffected by the application of nematicide. This phenomenon was more or less expected considering that, in contrast with nematodes and insects, fungi (including AMF) are not considered as a target for non-fumigant granular nematicides. On the contrary, use of a fumigant nematicide, such as methyl bromide, CH<sub>3</sub>BR, would have probably had a significant detrimental effect on root colonisation by AMF, since such chemicals have a potential to destroy all biological activity (Agrios, 1997). It is advised that any future studies on the AMF x potato x PCN x nematicide interaction or any attempt to incorporate AMF in integrated PCN

management programmes, should be carried out with granular rather than fumigant nematicides.

In contrast with aldicarb, *G. pallida* had a detrimental effect on root colonisation by AMF (significant PCN main effect), although AMF inoculation took place before PCN inoculation. One possible explanation for the reduction in mycorrhizal infection levels in PCN-infested roots is that nematode feeding and migration within the root cells might have disrupted to some extent the cortical parenchyma, thus reducing the available sites for fungus establishment and growth. Histological investigations of cortical tissues, which are the sites for both nematode development and mycorrhizal infection, using scanning electron microscopy (SEM) might be needed in future research to test this hypothesis. Scanning electron microscopes produce images of high resolution, which means that closely spaced features (i.e. nematodes and AMF structures in this case) can be examined more thoroughly compared to light microscopes. Examination of the structural changes in root cortical tissues by SEM might help clarifying the mechanisms involved in AMF x PCN interactions (e.g. competition for habitable space, nutrients; Hussey & Roncadori, 1978). The current results support the observation of O'Bannon & Nemec (1979) that root invasion by endoparasitic nematodes is destructive and likely to reduce the length of the root system colonised by mycorrhizal fungi. Ryan *et al.* (2000) also found that *G. pallida*-infested plants exhibited reduced percentage mycorrhizal root colonisation compared to plants without *G. pallida* at 4 and 6 wk after PCN inoculation, although the difference disappeared after 8 wk.

The three single AMF isolates did not exhibit better characteristics than the mixed-isolate inoculum with regard to potato root length colonisation. In particular, one of the single isolates (*G. dussii*) colonised only a quarter of the root length. The

levels of potato root colonisation by *G. intraradices* and *G. mosseae* were very much the same as those reported by McArthur & Knowles (1993) at 28 days after planting. Although root inoculation with Vaminoc resulted in significantly greater colonisation of the root system than the single isolates, it was apparent that the effects of the single isolates on root colonisation were not additive towards Vaminoc. Since the conditions under which the four AMF isolates were tested for their capacity to colonise the potato root were the same (e.g. soil, pH, environmental conditions), the differences in the degree of root colonisation among the four AMF were probably the result of structural and developmental differences between them, which may have consequently influenced their root colonisation characteristics. There is evidence in the literature to support this hypothesis. Using cucumber (*Cucumis sativus*) as a host plant, Larsen *et al.*, (2001), compared the root colonisation characteristics of *G. mosseae* and *Glomus geosporum* and observed significant differences in structural features (e.g. spore morphology and hyphal growth) and hence, in percentage root length colonisation between them. A better understanding of the dynamics of mycorrhizal hyphal growth during the symbiotic phase will be required in the future in order to understand the mechanisms involved in the formation of AMF mycelial networks and to explain the differences in the degree of root colonisation between different species of Glomalean fungi.

The *G. pallida* levels at harvest were higher than at planting in all treatments (with/without aldicarb, with/without AMF), apparently because of the low initial nematode population density (5 eggs g<sup>-1</sup> soil). Incorporation of aldicarb granules at planting to a depth of 15 cm at the commercial field rate proved effective, because PCN multiplication in nematicide treated pots was significantly lower than in untreated controls in both the presence and absence of AMF. Woods & Haydock



(2000) reported that granular nematicide incorporation to a medium depth of *c.* 15 cm gave a significantly more effective PCN control, compared to shallow (< 5 cm) or deep (> 35 cm) incorporation depths. The most interesting finding of the *G. pallida* multiplication studies was that AMF had a controlling effect on  $P_f/P_i$  not only in the presence but also in the absence of nematicide. It would have been expected that in pots not treated with nematicide, the increased hatch of *G. pallida* from mycorrhizal, as compared to non-mycorrhizal plants, would have led to an increase in the numbers of  $J_2$ s invading the root and hence, to an increase in the nematode multiplication. However, the  $P_f/P_i$  was lower in the four types of mycorrhizal plants than in the non-mycorrhizal plants in the absence of nematicide. In such case, the lower  $P_f/P_i$  exhibited under AMF-inoculated plants (relative to minus AMF plants) in the presence of nematicide, would have been expected to be just the result of the acceleration of the early hatch of *G. pallida* (associated with increased nematicide efficacy). The  $P_f/P_i$  results from the minus nematicide treatment, suggest that the enhancement of the positive effect of the nematicide on the *G. pallida* population change by AMF inoculation of potato roots may have not been simply the product of the AMF-induced stimulation of hatch as hypothesised, but more likely the result of a reduction in the number of nematodes entering, developing and subsequently, reproducing within mycorrhizal roots, compared to non-mycorrhizal roots. This hypothesis was supported by the observation that mycorrhizal plants allowed the development of significantly fewer fertilised females per plant compared to non-mycorrhizal controls. Although higher numbers of  $J_2$ s had been accumulated in mycorrhizal-treated soil and hence, attracted to the roots (relative to minus AMF soil/plants), as a result of the increase in hatch, the lower nematode multiplication on AMF-inoculated plants, compared to minus AMF plants, suggests that a large

number of hatched J<sub>2</sub>s under mycorrhizal plants might have been forced to starvation after failing to invade and multiply within the potato root. In the treatment involving nematicide, the reduced P<sub>f</sub>/P<sub>i</sub> on plus AMF, as compared to minus AMF plants, could be attributed in addition to starvation (due to mycorrhizal establishment in the roots) to an increase in nematicide efficacy within the first 4 wk from planting (due to the observed increase in *G. pallida* hatch). The fact that AMF inoculation took place before PCN inoculation might explain this controlling effect of AMF on the population increase of *G. pallida*. It was concluded that pre-inoculation of potato with AMF allowed the mycorrhizal fungi to become established in the roots before the introduction of PCN and consequently, resulted in fewer J<sub>2</sub>s penetrating and developing in roots of mycorrhizal plants than in roots of non-mycorrhizal plants. Similar results to these reported here were obtained by Hussey & Roncadori (1982), in a study looking on the effects of pre-inoculating tomato with *G. mosseae* on the reproduction of the root-knot nematode *Meloidogyne incognita*, although their study did not include nematicide.

It was apparent from the results of the dry biomass measurements that the single component isolates affected the potato plant growth differently than Vaminoc. A characteristic example was that of root dry weight in the absence of PCN and nematicide; although after 2 wk only Vaminoc-inoculated plants exhibited greater root dry weights than the non-mycorrhizal plants, at wk 4, this effect was shifted to the single AMF isolates. The percentage mycorrhizal colonisation of the root system did not appear to be positively associated with plant growth. In fact, 4 wk after planting, root and shoot dry mass of plants inoculated with the single AMF isolates was greater than in the Vaminoc-inoculated plants, despite the fact that the single isolates had colonised a lower percentage of root length than the Vaminoc mixture. A

similar observation was made by Pinochet *et al.*, (1998), who found that inoculation of plum rootstock (Myrobalan 29C) plants with *G. mosseae* resulted in significantly lower root colonisation than *G. intraradices*, but plants inoculated with *G. mosseae* had significantly increased fresh shoot and root mass than the *G. intraradices*-inoculated plants. The reason for this negative association between percentage colonisation and dry biomass observed here (in the absence of PCN and nematicide) after 4 wk for both root and shoot dry weights between Vaminoc- and single AMF-inoculated plants is not totally understood. In addition, literature information on the mode of action of AMF in specific plant-mycorrhizal symbiotic associations, which may have assisted in understanding the trends of the current study is very limited. However, it was evident that inoculation of roots of potato with the single isolates *G. intraradices*, *G. mosseae* and *G. dussii* had a better effect on potato plant growth than inoculation with the Vaminoc mixture. The two single factors PCN and nematicide had a significant main effect on both root and total dry weight, but they interacted significantly only towards shoot dry weight and total dry weight. Inoculation with PCN had a detrimental effect on total dry mass production only in the absence of nematicide, obviously because of the higher number of juveniles feeding in the roots of untreated compared to nematicide-treated plants. Aldicarb incorporation in the soil, as expected, eliminated the negative effect of *G. pallida* on plant growth. Interestingly, nematicidal application in the absence of PCN somehow hindered plant growth. This was probably the result of the elimination of the stimulatory effect of the single AMF isolates on shoot dry weight by the nematicide. The reason for this effect is not known.

The positive effect of the single AMF isolates on total dry mass production (in the absence of PCN and nematicide) was mirrored in the tuber yield data. The

significant main effect of AMF on tuber yield was the result of the significantly increased yield produced by the mycorrhizal plants (apart from those inoculated with Vaminoc), compared to non-mycorrhizal plants. From the four combinations of PCN x nematicide treatments, the only time that inoculation with AMF (single isolates) resulted in significant increases in tuber yield was in the minus PCN/minus nematicide treatment. As with the total dry mass it appeared that nematicide application limited the positive effects of the Vaminoc component isolates in tuber yield in the absence and presence of PCN. However, this finding, although interesting, is of limited importance since potato growers would not apply a nematicide if the field is free of PCN. The stimulatory effect of the single AMF isolates on tuber yield may have been the result of the altered production of one or more plant growth regulators (e.g. gibberellic acid) which can affect tuber growth and hence, yield.

In conclusion, the results of this experiment suggested that AMF isolates, pure or mixed, could offer a great prospect in the control of *G. pallida* primarily by controlling the nematode multiplication and therefore, improving the effectiveness of granular nematicides used in its control. The next step in this research will involve application of AMF in *G. pallida*-infested fields in the presence or absence of aldicarb. Confirmation of the results obtained here under field conditions, would open up new avenues on the management of *G. pallida*, such as AMF application as part of integrated PCN management systems.

## References

Agrios G N. 1997. *Plant Pathology*. San Diego, California, USA: Academic Press.

635 pp.

- Byrne J, Twomey U, Maher N, Devine K J, Jones P W. 1998.** Detection of hatching inhibitors and hatching factor stimulants for golden potato cyst nematode, *Globodera rostochiensis*, in potato root leachate. *Annals of Applied Biology* **132**:463-472.
- Carroll M. 1995.** *Characterisation of rhizosphere bacterial isolates which affect the hatch of potato cyst nematodes*. MSc Thesis, The National University of Ireland, Cork, Ireland. 70 pp.
- Deliopoulos T, Devine K J, Haydock P P J, Jones P W. 2004.** Studies on the effect of mycorrhization of potato roots on the hatching activity of potato root leachate towards the potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*. *Nematology* (submitted).
- Devine K J, Byrne J, Maher N, Jones P W. 1996.** Resolution of natural hatching factors for the golden potato cyst nematode, *Globodera rostochiensis*. *Annals of Applied Biology* **129**:323-334.
- Evans K, Brodie B B. 1980.** The origin and distribution of the golden nematode and its potential in the USA. *American Potato Journal* **57**:70-89.
- Evans S G, Wright D J. 1982.** Effects of the nematicide oxamyl on life cycle stages of *Globodera rostochiensis*. *Annals of Applied Biology* **100**:511-519.
- Giovannetti M, Mosse B. 1980.** An evaluation of techniques for measuring vesicular-arbuscular infection in roots. *New Phytologist* **84**:489-500.
- Hague N G M, Pain B F. 1970.** Some observations on the effect of 'Temik' on the potato cyst-eelworm, *Heterodera rostochiensis* Woll. *Plant Pathology* **19**:69-71.
- Hague N G M, Pain B F. 1973.** The effect of organophosphorus compounds and oxime carbamates on the potato cyst nematode *Heterodera rostochiensis* Woll. *Pesticide Science* **4**:459-465.

- Hussey R S, Roncadori R W. 1978.** Interactions of *Pratylenchus brachyurus* and *Gigaspora margarita* on cotton. *Journal of Nematology* **10**:16-20.
- Hussey R S, Roncadori R W. 1982.** Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. *Plant Disease* **16**:9-14.
- Ingham R E. 1988.** Interactions between nematodes and vesicular-arbuscular mycorrhizae. *Agriculture, Ecosystems and Environment* **24**:169-182.
- Jones P W, Tylka G L, Perry R N. 1998.** Hatching. In *The Physiology and Biochemistry of Free-living and Plant Parasitic Nematodes*, pp. 181-212. Eds R N Perry and D J Wright. Wallingford, UK: CAB International.
- Larsen J, Kjøller R, Rosendahl S. 2001.** Comparison of inter- and intraspecific colonization characteristics of *Glomus mosseae* and *Glomus geosporum* within the roots of *Cucumis sativus*. In *Knowledge on Population Biology of AMF as a tool for Mycorrhizal Technology*, Book of Abstracts. Eds J Rydlova *et al.* Cost 838 Action. Pruhonice, Czech Republic. 26-29 September 2001.
- McArthur D A J, Knowles N R. 1993.** Influence of species of vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition on growth, development, and mineral nutrition of potato (*Solanum tuberosum* L.). *Plant Physiology* **102**:771-782.
- Minnis S T, Haydock P P J, Ibrahim S K, Grove I G, Evans K, Russell M A. 2002.** Potato cyst nematodes in England and Wales - Occurrence and distribution. *Annals of Applied Biology* **140**:187-195.
- O'Bannon J H, Nemec S. 1979.** The response of *Citrus limon* seedlings to a symbiont, *Glomus etunicatus* and a pathogen, *Radopholus similis*. *Journal of Nematology* **11**:270-275.

- Osborne P. 1973.** The effect of aldicarb on the hatching of *Heterodera rostochiensis* larvae. *Nematologica* **19**:7-14.
- Perry R N. 1989.** Dormancy and hatching of nematode eggs. *Parasitology Today* **5**:337-383.
- Pinochet J, Camprubí A, Calvet C, Fernández C. 1998.** Inducing tolerance to the root-lesion nematode *Pratylenchus vulnus* by early mycorrhizal inoculation of micropropagated Myrobalan 29C plum rootstock. *Journal of the American Society for Horticultural Science* **123**:342-347.
- Ryan N A, Deliopoulos T, Jones P W, Haydock P P J. 2003.** Effects of a mixed-isolate mycorrhizal inoculum on the potato - potato cyst nematode interaction. *Annals of Applied Biology* **143**:111-119.
- Ryan N A, Duffy E M, Cassells A C, Jones P W. 2000.** The effect of mycorrhizal fungi on the hatch of potato cyst nematodes. *Applied Soil Ecology* **15**:233-240.
- Ryan N A, Jones P W. 2003a.** Effect of tuber-borne micro-organisms on hatching activity of potato root leachate towards potato cyst nematodes. *Nematology* **5**:55-63.
- Ryan N A, Jones P W. 2003b.** The effect of mycorrhization of potato roots on the hatching chemicals active towards the two potato cyst nematode species, *Globodera pallida* and *Globodera rostochiensis*. *Nematology* (in press).
- Smith G S. 1988.** The role of phosphorus nutrition in interactions of vesicular-arbuscular mycorrhizal fungi with soilborne nematodes and fungi. *Phytopathology* **78**:371-374.
- Tsutsumi M. 1976.** Conditions for collecting the potato root diffusate and the influence on the natural hatching of potato cyst nematode. *Japanese Journal of Nematology* **6**:10-13.

- Whitehead A G. 1992.** Emergence of juvenile potato cyst nematodes *Globodera rostochiensis* and *G. pallida* and the control of *G. pallida*. *Annals of Applied Biology* **120**:471-486.
- Whitehead A G. 1997.** Sedentary endoparasites of roots and tubers. In *Plant Nematode Control*, pp. 146-208. Ed. A G Whitehead. Wallingford, UK: CAB International.
- Winfield A L, Enfield M A, Foreman J H. 1987.** A column elutriator for extracting cyst nematodes and other small invertebrates from soil samples. *Annals of Applied Biology* **111**:213-231.
- Woods S R, Haydock P P J. 2000.** The effect of granular nematicide incorporation depth and potato planting depth on potatoes grown in land infested with the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Annals of Applied Biology* **136**:27-33.



## **Chapter F**

### **General Discussion**

## F.1 Hatch as a potential target for novel PCN control methods

Hatch is a critical stage in the PCN life cycle for nematode survival and Nordmeyer (1990) identified it as a possible target for the development of novel control agents. This is due to the near-absolute reliance of PCN on HF<sub>s</sub> produced in host root leachates to trigger hatch and to the limited viability of J<sub>2</sub>s in soil after they have hatched in the absence of the host plant (Section A.1.7). Consequently, any modification of the hatching activity of host root leachates towards PCN would be expected to have a large effect on PCN hatching behaviour. The PCN hatching mechanism has been extensively studied (Perry *et al.*, 1989; Perry, 1997; Jones *et al.*, 1998) opening up new prospects for PCN management using novel, hatch-based control methods. Prior to commencing this research, two such methods had been investigated with varying levels of success: a) stimulation of PCN hatch in the absence of host plants by exogenous application of HF<sub>s</sub> in soil ('suicide hatch'; Devine & Jones, 2000) and b) PCN hatch inhibition (Gonzalez *et al.*, 1994; Hackenberg & Sikora, 1994; Twomey, 1995; Byrne *et al.*, 1998; Perry *et al.*, 2000). These methods for controlling PCN may have great potential for incorporation in future IPM strategies, especially if the strict limitations set by the potato industry on nematicide usage, combined with the reduction in nematicide availability, continue (Haydock & Evans, 1998).

In contrast with *G. rostochiensis*, the traditional methods employed in PCN management, either separately or combined in IPM strategies (i.e. chemical control, resistant cultivars and rotation) have so far failed to control adequately the *G. pallida* population increases in the field for reasons that have already been discussed (Section A.1.7.3 and Table A.2, Chapter A). As a result, *G. pallida* is now the predominant species in many potato-growing regions, such as in the UK, where

potato growers face a serious *G. pallida* problem (Evans & Haydock, 2000). Because *G. pallida* hatches at a slower rate and over a longer period than *G. rostochiensis* (Evans, 1983; Whitehead, 1992) and the granular nematicides, such as aldicarb and oxamyl, have short half-lives (Whitehead, 1992; Ambrose *et al.*, 2000), by the time the peak of *G. pallida* hatch is reached, the nematicides may have already broken down to non-toxic levels in the soil and therefore be ineffective (Whitehead, 1992; Evans & Haydock, 2000). Acceleration of the hatch of *G. pallida* in the field so that it reaches peak levels before significant decay of the nematicide occurs, would therefore be expected to increase the effectiveness of these products.

## **F.2 Role of root colonising micro-organisms in PCN hatch**

The study of Devine *et al.* (1996), who found that root leachate from potato microplants grown under sterile conditions lacked several of the HFs produced in the PRL from conventionally grown plants, suggested that soil micro-organisms may play an important role in affecting PCN hatch by generating certain HFs in PRL. Evidence for a role for microbial HFs in PCN hatching was subsequently provided by Cronin *et al.* (1997) who isolated rhizobacteria capable of producing *G. rostochiensis*-active HFs. The accumulation of this information led to the speculation that mycorrhizal fungi, which are also root colonising micro-organisms with similar mode of action as rhizobacteria and which occupy regions of the root system similar to those colonised by PCN (Ingham, 1988), may also affect the capacity of potato plants to stimulate PCN hatch and/or be potential PCN biocontrol agents (both scenarios associated with increased nematicide effectiveness). Preliminary work conducted in the Department of Plant Science (University College Cork) to assess the effects of AMF on PCN hatch showed that inoculation of potato microplants cv.

Golden Wonder with Vaminoc, a commercial mixed-AMF inoculum consisting of three single-AMF isolates, stimulated the hatch of *G. pallida*, but not of *G. rostochiensis*, *in vitro* and in-soil (Ryan *et al.*, 2000). Although this was a promising initial step with regard to the proposed use of AMF in PCN control, a number of important parameters had to be considered and investigated before mycorrhization could be recommended as part of an integrated *G. pallida* control strategy. In light of this, a number of experiments were conducted in order to explore the various aspects of the potato-PCN-AMF-nematicide interaction.

### **F.3 Effects of AMF on PCN life cycle and on potato plant growth**

The first investigations focused on determining how AMF inoculation of potato affects other aspects of the PCN life cycle (nematode multiplication in particular) and plant growth, in addition to hatch. A pre-requisite of the use of AMF inoculation in PCN control would be that mycorrhization would have no deleterious effects on potato plant growth or yield characteristics because this would mask any positive effect it may have on controlling PCN. These issues were investigated by the experimental study presented in Chapter B, where the mixed-isolate Vaminoc inoculum was inoculated onto the roots of potato microplants cv. Golden Wonder.

Mycorrhization resulted in significant increases in the root and shoot dry weights of plants grown in the absence of PCN. Furthermore, Vaminoc-inoculated plants produced higher tuber yields than plants that did not receive mycorrhizal treatment. Inoculation of potato microplants with Vaminoc produced species-specific effects on the PCN life cycle, stimulating hatch of *G. pallida* but increasing the multiplication of *G. rostochiensis*. In particular, it was observed that mycorrhizal plants infested with *G. rostochiensis* produced 60% more cysts than their non-

mycorrhizal counterparts. Nevertheless, the root dry weight of plants inoculated with *G. rostochiensis* and Vaminoc, although less than the control plants (i.e. PCN non-infested non-mycorrhizal plants), was greater than in the treatment with *G. rostochiensis* (Fig. 2, Chapter B). This finding showed that inoculation of potato plants with Vaminoc compensated for part of the nematode damage sustained by the non-mycorrhizal plants. In other words, mycorrhization increased the tolerance of the plants to *G. rostochiensis*, something that was also manifested in the ability of Vaminoc-colonised plants to maintain high tuber yield in the face of PCN infestation. The phenomenon that damage to mycorrhizal plants infested with plant-parasitic nematodes can be lower than that of non-mycorrhizal nematode-infested plants has also been observed with respect to other nematode-AMF combinations. In cotton plants, for example, Hussey & Roncadori (1982) noted that the growth reduction due to the root-knot nematode *Meloidogyne incognita* was about 30% in non-mycorrhizal plants, but only 10% when plants were inoculated with the AMF species *Gigaspora margarita*.

In the experiment described in Chapter B, inoculation of potato microplants cv. Golden Wonder with Vaminoc caused a 14% increase in the in-sand hatch of *G. pallida* 2 weeks after planting (Table 1) which was a beneficial effect, in contrast with the increase in *G. rostochiensis* multiplication (Table 2) which was a detrimental effect (albeit associated with the beneficial effect of increased tolerance to *G. rostochiensis* as a result of increased root growth). The agricultural significance of these findings, if proved under field conditions, could be very high. In particular, it would mean that, unless a *G. rostochiensis*-resistant cultivar is grown, AMF inoculation of potato would probably benefit only crops that have pure or predominantly *G. pallida* population present.

That *G. rostochiensis* is better controlled in the UK than *G. pallida* by granular nematicides has already been discussed (Section A.1.10.4). Nevertheless, the use of nematicides to control *G. rostochiensis* remains an important component of PCN management, a situation likely to continue given the fact that, as shown by Minnis *et al.* (2002), *G. rostochiensis* is present in almost one-third of PCN-infested fields in England and Wales, in spite of the greater effectiveness of PCN control measures against this species, compared to *G. pallida*. In general, nematicides are more effective at PCN population control when used against low rather than high population densities because of the greater nematode multiplication rates in the former case (Haydock & Evans, 1998). In practical terms, this means that if the initial PCN population density ( $P_i$ ) is high, then the nematicide will be more effective at reducing the yield loss that would occur in the absence of nematicide than controlling the nematode population increase (Evans & Haydock, 2000). Consequently, a potential further increase in *G. rostochiensis* levels in the field following application of AMF may have adverse effects on long-term *G. rostochiensis* control if susceptible cultivars are grown (36% of potato cultivars grown in the UK are susceptible to both PCN species; Table A.7, Chapter A) and the pre-planting nematode densities are high. Due to the significance of these parameters in relation to the proposed use of AMF in PCN control it was important to expand the research and to repeat parts of the work described in Chapter B in multiple sites and under conditions that mimic the field situation in order to confirm the stability of the interactions reported.

The PCN species-specificity of the effect of AMF inoculation and the potential impact that this effect may have in the implementation and effectiveness of control measures against the two PCN species highlights the requirement for correct PCN

species identification (Section A.1.5) before any nematicide/AMF applications in future field studies of this nature. Recent development in the area includes work conducted by researchers in the Crop and Environment Research Centre (Harper Adams University College) who evaluated three widely used techniques in PCN diagnosis (PCR, IEF and ELISA) for achieving a rapid and accurate identification and quantification of the two PCN species from field soil samples (Ibrahim *et al.*, 2001).

The stimulatory effect of mycorrhization on root growth of potato cv. Golden Wonder in the absence of PCN was confirmed in the glasshouse experiment (Table 1, Chapter D) and the outdoor pot trial (Fig. 1, Chapter E); consistency of AMF effects between experiments was also observed with regard to fresh tuber yield in the absence of PCN (Chapter B, and Table 3, Chapter E). This consistency of results between experiments (also with regard to the effect of AMF on hatch; Section F.5) supports the veracity of the findings. That mycorrhization had an enhancing effect (in absence of PCN) or compensatory effect (in presence of PCN) on plant productivity was a positive outcome with agricultural and possibly ecological implications, such as increased tolerance to PCN attack, lower fertilisation input, more profitable yields and reduced use of agrochemicals.

In contrast with the underground plant parts, inoculation of potato cv. Golden Wonder with AMF did not equally stimulate haulm growth. In particular, while inoculation with all three AMF increased the root dry weight of plants, none of them increased leaf dry weight (Table 1, Chapter D). In potato, root growth and haulm growth are correlated, as, after shoot emergence, roots and haulm develop simultaneously; haulm and tuber growth also proceed simultaneously during part of

the growing period (Beukema & Van Der Zaag, 1990). It was therefore apparent that mycorrhizal activity in roots somehow altered the growing pattern of the plant.

The four main factors determining tuber production are photosynthesis, respiration, partitioning or distribution of assimilates, and dry matter content of tubers (Beukema & Van Der Zaag, 1990). The fact that root colonisation by AMF had no effect on leaf characters (i.e. total leaf area, chlorophyll content, leaf dry weight; Table 1, Chapter D) suggests that the foliage of AMF-inoculated and un-inoculated plants probably produced similar amounts of assimilates. In potato plants, uptake and assimilation of mineral nutrients is positively correlated with P nutrition, which, in turn, is critical for tuber development (McArthur & Knowles, 1993). Mycorrhizal fungi are known to increase plant nutrient supply (P in particular) and hence, to promote plant growth by: (a) acquiring nutrient forms that are normally unavailable to the plants, and (b) by extending the volume of soil accessible to the plants (in other words, increasing the surface area of the plant's root system) (Brundrett *et al.*, 1996). According to this, in well-fed plants with restricted root space, as in the glasshouse experiment presented in Chapter D, there would be no advantage to the plant of AMF-induced P gathering. In the same study, the observation that AMF-inoculated plants exhibited greater root dry weights than did the un-inoculated plants suggests increased transport of assimilates from the sink (i.e. leaves) to the source (initially roots and some weeks later, tubers) in the former. The consequence of this would be that more assimilates would be used for tuber growth and stored as starch in tubers in AMF-inoculated plants than in un-inoculated plants. The increase in tuber yield following mycorrhization of potato roots (Chapter B, and Table 3, Chapter E) supports this hypothesis. The knowledge that natural plant hormones contribute substantially to the distribution pattern of dry matter in plants



(including potato; Beukema & Van Der Zaag, 1990), and the findings of this research suggest that AMF have a great potential to alter the potato plant's hormonal balance (e.g. by inhibiting or reducing gibberellic acid, known to favour haulm growth at the expense of tuber growth). Direct evidence has been published demonstrating that AMF effects on plant development may be totally unrelated to plant mineral physiology (Smith & Gianinazzi-Pearson, 1988).

The results from the glasshouse experiment presented in Chapter C revealed differential effects of the four AMF treatments on plant growth parameters of most cultivars (Table 2, Chapter C) and also a high level of variability in the growth response of the potato cultivars to inoculation with each AMF. As a result, certain AMF-cultivar associations were more effective at promoting plant growth than others. The mycorrhizal fungus-cultivar specificity observed in this experiment is often observed in studies of AMF-cultivar interactions (examples are discussed in Chapter C). Fortuna *et al.* (1992) interpreted this phenomenon as a kind of functional host specificity. Although it is generally accepted that arbuscular mycorrhizal associations lack specificity, the degree of mycorrhizal dependency (Section A.2.3 and Section F.3.2) of the same plant may vary considerably with the fungal species present (Vestberg & Estaún, 1994).

#### **F.4 Mycorrhizal dependency of potato**

Part of the difficulty in making generalisations about AMF-host interactions is related to the inconsistency between experiments regarding the parameters assessed. For example some studies report aboveground (fresh or dry) plant mass, while others report underground plant mass or seed (or fruit) yield only. Usually, the decision as to which growth parameters will be assessed is governed by the specific aims of the

investigation. For example, if the aim of mycorrhization is to improve yield, then this may be the only parameter recorded. In AMF studies that are agriculturally orientated, as was the case with the research described here, rarely is the total plant dry mass assessed. When aiming to establish the overall benefit provided to the plant by AMF inoculation it seems better to express the result on the basis of total dry mass than calculating and presenting aboveground and underground dry mass separately. This would facilitate a more direct comparison of the growth responses of different plants to AMF inoculation. The relationship between the total dry mass of AMF-inoculated plants (a) and the total dry mass of un-inoculated plants (b) is the 'mycorrhizal dependency' (MD) of a plant (Plenchette *et al.*, 1983);  $MD = 1 - (b/a)$ . If MD is greater than zero it means that the plants were benefited from AMF inoculation.

In an attempt to quantify the amount of benefit provided to the seven potato cultivars by inoculation with AMF, it was decided to calculate the MD. For this purpose, data from leaf, shoot and root dry weights (Table 2, Chapter C, and Table 1, Chapter D) were combined to give the total dry mass of AMF-inoculated and un-inoculated plants. According to Van Der Heijden *et al.* (1998), due to the high level of variability often observed in the response of a plant species to different AMF treatments, it is preferable, where possible, to measure the MD of plants based on their response to more than one AMF, as this would allow a more meaningful interpretation of MD values. Therefore, it was decided to pool the data from the three granular AMF treatments Vaminoc, *G. intraradices* and *G. mosseae* in the calculation of total dry mass for AMF-inoculated plants.

The degree of MD varied greatly among the seven potato cultivars. The cultivars Home Guard and Golden Wonder benefited the most from AMF inoculation

(MD were 0.19 and 0.16, respectively). On the contrary, inoculation with AMF had a detrimental effect on the growth of potato cv. Maris Piper (MD = -0.57) and a very small effect on the growth of the other four cultivars (MD was 0.08, 0.06, 0.06 and -0.09 for cvs Saturna, British Queen, Pentland Dell and Bintje, respectively). That cv. Golden Wonder benefited from AMF inoculation was supported by the results from the studies of Chapters B and E, where AMF-colonised plants exhibited higher yields than non-AMF plants.

The observation that even cultivars with similar maturity levels, such as Maris Piper and Golden Wonder, differed so greatly in their MD, while others with different maturity levels exhibited similar MD values (e.g. British Queen and Pentland Dell) suggests that in potato, the maturity level of a cultivar seems not to be a determinant for the MD. In natural ecosystems, differences in MD of plant species, which is the rule rather than the exception, are particularly important in plant succession; plants with MD approaching unity (i.e. obligatory mycorrhizal) will not survive without being associated with AMF (Janos, 1980). In the study described here, none of the seven potato cultivars exhibited such a high MD value, namely they were not highly dependent on AMF for their growth. In other crops, such as maize, it is well established that AMF colonisation causes enhanced growth over uninoculated plants (Osnubi, 1994). Intensive agronomical crop systems have a tendency to eradicate native (indigenous) mycorrhizal inoculum (Brundrett *et al.*, 1996). Potato is a crop that requires intensive soil preparation prior to planting, and practices such as soil tillage, seed bed preparation and ridging would be expected to suppress any native AMF populations.

Various studies have been published on the potato-AMF interaction (Louche-Tessandier *et al.*, 1999; Duffy & Cassells, 2000; Ryan *et al.*, 2000), but all of them

involved AMF inoculation of micropropagated plantlets of a single potato cultivar. The use of tubers and of several potato cultivars in the same experimental study clearly differentiated this research from previous work in this area.

## **F.5 Effects of mycorrhization on the hatch of the two PCN species**

The observation that the Vaminoc-induced stimulatory effect on hatch was restricted to *G. pallida* (Chapter B) suggested that this effect may not have been simply the result of the larger root systems (the principal site of leaching of PCN hatching chemicals) supported by the mycorrhizal plants as compared to the non-mycorrhizal plants; in such case it would have been expected that AMF colonisation of the roots would induce a similar increase in the hatch of *G. rostochiensis*. Two scenarios were proposed to explain the stimulatory effect of mycorrhization on *G. pallida* hatch: (I) production of novel hatching chemicals by the fungal part of the AMF symbiosis, and (II) increased production of natural hatching chemicals (HFs and/or HSs; Section A.1.7.2.2) in PRL as a result of AMF colonisation of potato roots; these chemicals are known to exhibit PCN species-specificity and species-selectivity (Byrne, 1997, Byrne *et al.*, 2001).

To investigate scenario (I) a separate experiment was conducted, in which the same AMF inoculum, Vaminoc, was inoculated onto the roots of the non HF-producing (i.e. PCN non-host) wheat plants inoculated with *G. pallida* cysts (Chapter C). The results indicated that, in contrast with the potato experiment (Chapter B), Vaminoc inoculation of wheat had no significant effect on the hatch of *G. pallida*, indicating that the mycorrhizal effect on hatch was host (potato)-specific. Using the PCN non-host plant strawberry, Ryan *et al.* (2000) also found no evidence of

Vaminoc-induced stimulation of *G. pallida* hatch. These findings suggest that only solanaceous species may produce a stimulant which induces fungal HF production.

Another experiment was therefore designed (Chapter D) to assess whether the Vaminoc effect on hatch was due to altered production of HFs in PRL (i.e. scenario II); PRL from AMF-inoculated and un-inoculated plants was fractionated and the hatching activities of leachates from both sets of plants towards the two PCN species were assessed and compared (Table 4, Chapter D). The fact that PRL from Vaminoc-inoculated plants was found to contain greater quantities of HFs with activity towards *G. pallida* than the PRL from un-inoculated plants, in combination with the significant increase in the activity of several individual HFs compared to that in the PRL from un-inoculated plants, demonstrated that the speculation of Devine *et al.* (1996) of a possible microbial influence in the PCN hatching mechanism was correct. That bacteria isolated from the potato rhizosphere have a hatch-enhancing effect on *G. pallida* has already been demonstrated by Carroll (1995) and more recently by Ryan & Jones (2003), but the research presented in Chapter C is the first experimental evidence for a similar role for AMF in affecting HF production in PRL.

When the results from the *in vitro* experiment (Table 2, Chapter D) were compared with those from the in-sand study (Table 1, Chapter B), many similarities were observed. In the presence of potato plants cv. Golden Wonder, *G. pallida* hatch was significantly less than that of *G. rostochiensis* in the first 2 or 3 weeks from planting. When plants were inoculated with AMF, the hatch of *G. pallida* was increased relative to un-inoculated plants, although it was still less than that of *G. rostochiensis*. In the absence of AMF, other authors (Evans, 1983; Whitehead, 1992) reported that *G. pallida* hatches slower than *G. rostochiensis*. However, the results from this research suggest that there may be a great potential to minimise the

difference between *G. pallida* and *G. rostochiensis* hatch in soils with mixed-species infestations, by inoculating potato with AMF. That AMF colonisation of potato roots decreased significantly the *G. rostochiensis* : *G. pallida* hatch ratio in the first 3 weeks from planting was also demonstrated in the multi-cultivar study presented in Chapter C (Fig. 1).

Direct evidence has been published indicating that the hatching activity of PRL towards the two PCN species varies with the cultivar used (Turner & Stone, 1981; Evans, 1983). The research described in Chapter B, as well as that of Ryan *et al.* (2000), involved a single potato cultivar, Golden Wonder, which is susceptible to both PCN species. One of the criteria for AMF-accelerated *G. pallida* hatch to be used as part of an integrated PCN control plan in commercial potato crops is the stimulation of high levels of nematode hatch over a wide range of potato cultivars exhibiting different characteristics, such as levels of PCN resistance, plant growth patterns and/or maturity levels. Stimulation of *G. pallida* hatch in the field in the presence of a PCN resistant cultivar would expect to achieve better control of this pest, compared to that in the presence of a susceptible cultivar. In the UK, potato cv. Santé has been reported to reduce *G. pallida* multiplication by as much as 88% (Whitehead, 1991); if the AMF stimulatory effect on hatch is cultivar-independent, then the use of a cultivar like 'Santé' together with one application of AMF and one of a granular nematicide may suppress the population of *G. pallida* to such low levels that the subsequent crop will not be at risk. An experiment was therefore designed (Chapter C) to assess whether the AMF effects on hatch are cultivar-independent. The main conclusions from this study could be summarised as the following: (a) inoculation of potato roots of all cultivars with granular formulations of AMF inocula induced a significant increase in the *in vitro* hatch of *G. pallida* from 3-week-

old potato plants; (b) *G. rostochiensis* hatch was generally less affected by mycorrhization compared to *G. pallida*; (c) despite the significant stimulatory effect that mycorrhization had on the hatch of *G. pallida*, the hatch of *G. rostochiensis* was still higher than *G. pallida*; (d) the Vaminoc component isolates, *G. intraradices* and *G. mosseae*, did not exhibit better characteristics than Vaminoc on *G. pallida* hatch; and (e) inoculation with *Gi. rosea* had no effect on hatch of either PCN species, possibly due to the low percentage of root length colonised by this AMF, and for this reason it was withdrawn from further study.

In those three experiments (Chapters B, C and D) where *G. rostochiensis* hatching behaviour was monitored under young potato plants (i.e. 2 or 3-week old), percentage hatch was consistently greater than 80%, demonstrating that *G. rostochiensis* hatch had reached a maximum in the first 2 to 3 weeks from planting. These findings were in full agreement with those reported in the field by LaMondia & Brodie (1986). The consequence of *G. rostochiensis* hatch possibly reaching its peak within the first 3 weeks from planting in the absence of AMF was that the increase in HF production following mycorrhization of plants with Vaminoc or with the single-AMF isolates (Table 4, Chapter D) could not result in increasing the total *in vitro* hatch (Table 2, Chapter D). In a subsequent study conducted in this laboratory to investigate whether inoculation of potato microplants with Vaminoc affects, in addition to HFs, the production of other hatching chemicals in PRL (i.e. HSs and/or HIs), Ryan & Jones (2003) reported a similar situation with regard to the hatching behaviour of *G. rostochiensis*; percentage hatch was similar in the unfractionated PRL from Vaminoc-inoculated and un-inoculated plants, but when these leachates were fractionated, PRL from Vaminoc-inoculated plants was found to contain greater quantities of HFs compared to the PRL from un-inoculated plants.

With regard to *G. pallida* hatch, the authors confirmed that colonisation of potato roots with Vaminoc stimulated HF production. The same study also demonstrated that PRL from Vaminoc-inoculated plants contained markedly more HSs active towards *G. pallida* than the PRL from un-inoculated plants, but no differences in HI levels between the two PRL profiles were detected. In the study presented in Chapter D, the increase in *G. pallida* hatch following inoculation of roots with *G. mosseae* was not attributed to increased HF production due to fungal activity in roots. Nevertheless, the findings of Ryan & Jones (2003) and the knowledge that *G. mosseae* is part of the Vaminoc mixture suggest that it may be possible that the stimulatory effect of *G. mosseae* on *G. pallida* hatch in the un-fractionated PRL was the result of an increase in the quantity and/or activity of HSs in the PRL. To investigate this possibility, PRL from plants inoculated with *G. mosseae* would need to be fractionated and assayed for HSs. In the study of Chapter D, the *in vitro* hatch of *G. pallida* from 3-week-old *G. intraradices*-inoculated plants was statistically similar with that from un-inoculated plants (Table 2) and there were no quantitative differences in HF production between the two PRL profiles (Table 4). However, combination of data from all three experiments where the effect of *G. intraradices* on PCN hatch was monitored (i.e. Fig. 1, Chapter C; Table 2, Chapter D; Table 1, Chapter E) revealed that, overall, colonisation of potato roots by *G. intraradices* caused a significant increase in total *G. pallida* hatch (relative to un-inoculated plants) in the first 3-4 weeks after planting. The possibility, therefore, that *G. intraradices* activity in potato roots stimulates the production of HSs in PRL cannot be ruled out and merits future investigation.

In an attempt to draw together the findings of the individual chapters with regard to PCN hatch, it was decided to pool the results from the three experiments



where the hatching behaviour of both species was observed (2- and 3-week-old plants only, i.e. Table 1, Chapter B; Fig. 1, Chapter C; Table 2, Chapter D). The combined result simply confirmed what has already been discussed in the individual chapters, that mycorrhization caused a significant increase in the hatch of *G. pallida* (combined grand means were 59% and 76% for non-AMF and AMF-inoculated plants, respectively) and that the hatch of *G. rostochiensis* from non-AMF plants (mean of 83%) and AMF-inoculated plants (87%) was statistically similar.

Several authors have published studies of comparisons of PCN hatching responses to PRL from different host genotypes (Turner & Stone, 1981; Evans, 1983; Farrer & Phillips, 1983; Forrest & Phillips, 1984) but evaluating the significance of their findings is difficult because of inter-genotype differences in root growth and root development characteristics. The novelty of the method followed in the experiment presented in Chapter C to compare the PCN hatching responses to the different PRL preparations (i.e. mycorrhizal vs. non-mycorrhizal) was the elimination of differences in root development characteristics. This was achieved by standardising the leachates on the basis of their carbon content, thus allowing direct comparison of PRL hatching activities. That PRL dry weight is closely correlated with carbon content has been shown by Devine & Jones (2001). Since the root system is the major site of leaching of hatching chemicals from the host plant, it is expected that differences in root system size and more importantly, root branching patterns would have a large effect on PRL production and hence, on the hatching activity of the leachate (Rawsthorne & Brodie, 1986).

## F.6 Comparative effectiveness of mixed- and single-AMF inocula

As already mentioned, Vaminoc is a commercial mixture of three selected isolates of *Glomus* spp. (*G. intraradices*-BioRize BB-E, *G. mosseae*-BEG 12, and *G. dussii*-BioRize BB-Scl; BioRize, Dijon, France). Direct evidence has been published indicating that inter-specific differences in structural and developmental features of *Glomus* species have a large effect on root colonisation characteristics (Larsen *et al.*, 2001) and potentially, on plant growth. There is therefore a possibility that inoculation of potato with the Vaminoc component species-isolates (or perhaps with other AMF) would have different effects than Vaminoc on parameters such as root length colonisation, plant growth, PCN hatch and multiplication, and HF production in PRL. In light of this, it was decided to test for the first time, in addition to Vaminoc, the effects of single-AMF isolates on the potato-PCN interaction (Chapters C, D and E).

Following consideration of the findings described in Chapter B and in order to determine if these various effects of Vaminoc on PCN life cycle were intrinsically interconnected (i.e. the early hatch of *G. pallida* was causally related to the high multiplication of *G. rostochiensis*) it was decided to inoculate the same cultivar, Golden Wonder, with the Vaminoc component isolates, *G. intraradices* and *G. mosseae* (Chapter D). The observations of the study in Chapter B lead to the hypothesis that it may be possible to separate the positive effects of Vaminoc from the negative ones, by replacing the mixed-inoculum Vaminoc with one of its component isolates, if these had distinct and separate effects on the PCN life cycle when used separately (i.e. one isolate causing the hatch effect and the other one the root growth and hence, tolerance and multiplication effects). However, this prospect was later dismissed as unfeasible because the results obtained in the glasshouse

experiment in Chapter D revealed stimulatory effects on root growth by both isolates (Table 1) but a hatch-enhancing effect only by one of the isolates, *G. mosseae* (Table 2).

It was unfortunate that at the time of conducting the experiments of Chapters C and D, the third component of the Vaminoc mixture, that is *G. dussii*, was not available in pure formulation in order to test the effects of inoculation of potato with this AMF isolate on the various aspects of the potato-PCN interaction compared to Vaminoc. As soon as *G. dussii* inoculum became available in a pure formulation it was decided to include it in the research (outdoor pot trial; Chapter E). Despite the significantly lower level of root colonisation achieved by inoculation with *G. dussii*, as compared to the other two single-AMF isolates, colonisation by *G. dussii* also resulted in stimulatory effects on *G. pallida* hatch and root growth (significant AMF main effect; Table 1 and Fig. 1, respectively, Chapter E), confirming that replacement of Vaminoc with the individual component isolates could not break the nexus of beneficial and detrimental effects of Vaminoc inoculation.

AMF-inoculated potato plants were evaluated for percentage root length colonisation at various phases of their development (2, 4, 6 and 12 weeks after planting or shoot emergence, depending on the experiment). Successful mycorrhizal colonisation was confirmed from 2 weeks onwards (Chapter B), and was observed for both micropropagated plants and seed-tuber-derived plants (all seven cultivars) growing in either sterile (Chapters B, C and D) or non-sterile (Chapter E) growth substrate. Nevertheless, the five AMF tested for their ability to colonise the potato root system exhibited significant differences in percentage colonisation in the order: Vaminoc > *G. intraradices* = *G. mosseae* > *G. dussii* > *Gi. rosea*. Direct comparison of data on AMF root colonisation for cv. Golden Wonder between the in-sand and in-

soil experiments (Chapters D and E) showed that the use of non-sterile growth substrate resulted in substantial reductions in the length of root system colonised by all three AMF inocula trialled. This reduction in mycorrhizal colonisation could be attributed to competition of AMF with other soil microbes for habitable space within the potato roots, which may have resulted in reducing the amount of available root tissue for AMF growth. Since AMF and other soil biota share ecological niches and exploit the same carbohydrate resource, it is logical to consider an interaction between them, which may be either beneficial or deleterious for AMF (Larsen & Ravnskov, 2001).

The effectiveness of inoculation with single-AMF isolates vs. Vaminoc on PCN hatch in standard and fractionated PRL has already been discussed in Section F.5. Similarly, the AMF effects on plant growth have been described in all experimental chapters. In general, there was very little variation among the three most studied AMF (i.e. Vaminoc, *G. intraradices* and *G. mosseae*) with regard to plant growth parameters. A closer examination of data in Table 2, Chapter C, and Table 1, Chapter D revealed that in nearly 90% of the cases (112 out of 126 comparisons; 3 AMF x 7 potato cultivars x 6 plant characters) the three AMF had a similar effect on plant growth parameters, although, as explained in Section F.4, measurements and interpretations of MD values based solely on the response to one AMF are of little ecological significance and should be avoided. Nevertheless, for the purpose of comparing the mixed-AMF with the single-AMF isolates with regard to their effects on plant growth, it was decided to calculate MD for each AMF separately; MD of potato based on Vaminoc was 0.055, on *G. intraradices* 0.001, and on *G. mosseae* 0.012. These results clearly demonstrated that mycorrhization had no negative effects on plant development, which is a promising aspect with regard to

its proposed use in integrated *G. pallida* control, indicating that there would be no undesirable effects from AMF inoculation on plant productivity and subsequently, yield.

## **F.7 Potential of AMF for field applications - role of nematicides**

The findings of the experiments presented in Chapters B, C and D provided the first detailed characterisation of the complex interactions between potato plants, commercial AMF inocula and the two PCN species. The factors governing the potato-PCN interaction were already known prior to commencing this research, but these studies provided clarification of how AMF influence this interaction. It was concluded that the key events taking place following application of AMF inocula onto potato roots are: (a) AMF starts colonising the potato root rapidly after inoculation; as a result, (b) the fungus alters HF production in the PRL within the first 3 weeks from plant emergence; (c) this induces significant increases in *G. pallida* hatch and some stimulation of root growth; at the same time, (d) haulm growth remains largely unaffected by the mycorrhizal activity but tuber yield may be increased.

The last step before conducting field trials to assess AMF as an element of an integrated *G. pallida* control strategy was to investigate the compatibility of AMF with the granular nematicide aldicarb. In light of this, a separate experiment was conducted based on seed-tuber-derived plants (cv. Golden Wonder) grown outdoors in pots (Chapter E). In addition to the inclusion of a nematicide, an important parameter that differentiated this study from the experiments described in the preceding chapters (B, C and D) was the use of non-sterile soil, which would be the material of choice for field experimentation.

A promising aspect of using AMF was that nematicide application did not affect the colonisation potential of the mycorrhizal fungus. In addition to the hatch-enhancing effect, other positive results were demonstrated by mycorrhization of potato roots. Pre-inoculation of sprouted tubers with AMF, followed by a second application after 2 weeks (when PCN inoculum was added to the pots), had a controlling effect on *G. pallida* population increase in the absence of the nematicide (Table 2, Chapter E). Furthermore, it increased the effectiveness of nematicide by almost halving the nematode multiplication rate in the presence of aldicarb (Table 2, Chapter E). These results suggest that AMF have a great potential to be used as *G. pallida* biocontrol agents.

Several studies have been published describing interactions between AMF and plant-parasitic nematodes (Section A.2.5) but the experiment described in Chapter E is the first such study conducted with *G. pallida*. The effect of host plant-nematode-AMF interaction may be adverse, neutral or stimulatory for the nematode (Table A.12, Chapter A). There are several possible ways by which AMF could control root pathogens, including PCN, and detailed reviews summarising and discussing results on AMF and biological control can be found in Dehne (1982), Smith (1987) and Linderman (1988). In most of these published cases, prior colonisation by AMF was necessary for protection against nematodes.

In commercial potato production, tubers require a pre-sprouting period, as this results in an early crop, early emergence, early tuber initiation and hence, high yields. Tubers are then planted when they have developed strong and firm sprouts (about 2 cm long) before roots start to develop (Beukema & Van Der Zaag, 1990). The lack of developed roots for AMF inoculation may be not a problem if the inoculum would be applied just below the sprouted tuber at planting. This is because,

for pre-sprouted tubers, root formation starts immediately after planting (Beukema & Van Der Zaag, 1990), therefore guaranteeing a rapid contact between AMF propagules and developing roots, which is necessary for the symbiosis to be established (Section A.2.2). For these reasons, IPM control plans that would include AMF should be based on pre-sprouted tubers rather than on un-sprouted tubers planted straight from cold storage. Although AMF were shown to be compatible with the aldicarb nematicide in this study (level of root colonisation by AMF was not reduced by the nematicide), a factor that would possibly limit their effectiveness in commercial potato production is their incompatibility with high fertilisation, particularly of phosphorus (Hayman, 1983), and with the intensive agricultural practices (Brundrett *et al.*, 1996). Mycorrhizal establishment may be also prevented by certain fungicides (Kjølner & Rosendahl, 2000). Another problem with the use of AMF in the field is associated with the increased amount of inoculum needed for an effective control; commercial AMF inocula are very expensive to be used in a large field-scale. There is therefore an urgent requirement to produce AMF inocula that are highly effective in small quantities. Mycorrhizal fungi are root-colonising micro-organisms and therefore, care should be taken that the AMF inoculum is applied as close to the root zone as possible, because failure to do so, would result in substantial loss of mycorrhizal activity. Possible methods of field applications of AMF have been presented in Section A.2.4.2.

Although nowadays there is an abundance of commercial AMF inocula, their utilisation at commercial level is limited due to their general incompatibility with pesticides and fertilisers. Therefore, the optimal conditions compatible with their utilisation should be determined beforehand. The French company BioRize, apart from producing a wide range of AMF inocula, has produced fertilisers, such as

MycoFert (a mineral fertiliser) and OrgaFert (an organic fertiliser) that are well adapted to AMF inocula. MicroBio Division in Royston, Herts, UK, also produces AMF in a commercial scale.

## **F.8 Future research**

This research concludes that there is a great potential for improving substantially the result of granular nematicide applications on the control of *G. pallida* by applying selected AMF inocula in the soil at the time of planting potatoes. The complex aspects of the tritrophic (potato-PCN-AMF) interaction are now better understood, and the use of AMF in integrated *G. pallida* management programmes is strongly recommended.

Recommendations for future research:

- test the effects of AMF inoculation on PCN hatch, nematode multiplication and tuber yield in the field,
- investigate the compatibility of AMF inocula with the other granular nematicide used in the UK, e.g. oxamyl,
- test the effects of AMF over a range of soil fertility levels, and in the presence of common fungicides,
- optimise the method of AMF application for best results,
- investigate the interactions between PCN, AMF and nutrient availability,
- assess the effects of the other root colonising microorganisms, such as rhizobacteria, on the PCN hatch in the field,
- use scanning electron microscopy to examine nematode development and mycorrhizal infection simultaneously,



- use stable isotope discrimination to identify the source (i.e. plant or fungus) of HFs produced in PRL from mycorrhizal and non-mycorrhizal plants,
- conduct field applications of large volumes of PRL collected from AMF-colonised plants to a fallow soil or to a field planted to a non-host crop to induce PCN hatch, following which the juvenile nematodes die of starvation (i.e. 'suicide' hatch strategy).

## F.9 References

- AMBROSE, E., HAYDOCK, P.P.J. & WILCOX, A. (2000). Degradation of the nematicide oxamyl in field conditions. *Aspects of Applied Biology* 59, 41-51.
- BEUKEMA, H.P. & VAN DER ZAAG, D.E. (1990). Introduction to Potato Production. Pudoc, Wageningen, The Netherlands, 208 pp.
- BRUNDRETT, M.C., BOUGHER, N., DELL, B., GROVE, T. & MALAJCZUK, N. (1996). *Working with mycorrhizas in forestry and agriculture*. ACIAR Monograph 32, Australian Centre for International Agricultural Research, Canberra, Australia, 374 pp.
- BYRNE, J. (1997). *Comparative hatching behaviour of Globodera rostochiensis and G. pallida*. Ph.D. Thesis, The National University of Ireland, Cork, Ireland, 302 pp.
- BYRNE, J., MAHER, N.J. & JONES, P.W. (2001). Comparative responses of *Globodera rostochiensis* and *G. pallida* to hatching chemicals. *Journal of Nematology* 33, 195-202.
- BYRNE, J., TWOMEY, U., MAHER, N., DEVINE, K.J. & JONES, P.W. (1998). Detection of hatching inhibitors and hatching factor stimulants for golden potato cyst nematode, *Globodera rostochiensis*, in potato root leachate. *Annals of Applied Biology* 132, 463-472.

- CARROLL, M. (1995). Characterisation of rhizosphere bacterial isolates which affect the hatch of potato cyst nematodes. MSc thesis, The National University of Ireland, Cork, Ireland, 70 pp.
- CRONIN, D., MOENNE-LOCCOZ, Y., FENTON, A., DUNNE, C.C., DOWLING, D.N. & O'GARA, F. (1997). Role of 2,4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. *Applied and Environmental Microbiology* 6, 1357-1361.
- DEHNE, H.W. (1982). Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology* 72, 1115-1119.
- DEVINE, K.J., BYRNE, J., MAHER, N. & JONES, P.W. (1996). Resolution of natural hatching factors for the golden potato cyst nematode, *Globodera rostochiensis*. *Annals of Applied Biology* 129, 323-334.
- DEVINE, K.J. & JONES, P.W. (2000). Response of *Globodera rostochiensis* to exogenously applied hatching factors in soil. *Annals of Applied Biology* 137, 21-29.
- DEVINE, K.J. & JONES, P.W. (2001). Potato cyst nematode hatching activity and hatching factors in inter-specific *Solanum* hybrids. *Nematology* 3, 141-149.
- DUFFY, E.M. & CASSELLS, A.C. (2000). The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. *Applied Soil Ecology* 15, 137-144.
- EVANS, K. (1983). Hatching of potato cyst nematodes in root diffusates collected from twenty-five potato cultivars. *Crop Protection* 2, 97-103.
- EVANS, K. & HAYDOCK, P.P.J. (2000). Potato cyst nematode management - present and future. *Aspects of Applied Biology* 59, 91-97.
- FARRER, L.A. & PHILLIPS, M.S. (1983). *In vitro* hatching of *Globodera pallida* in response to *Solanum vernei* and *S. tuberosum* x *S. vernei* hybrids. *Revue de Nématologie* 6, 165-169.

- FORREST, J.M.S. & PHILLIPS, M.S. (1984). The effect of *Solanum tuberosum* x *S. vernei* hybrids on hatching of the potato cyst nematode *Globodera pallida*. *Annals of Applied Biology* 104, 521-526.
- FORTUNA, P., CITERNESI, S., MORINI, S., GIOVANNETTI, M. & LORETI, F. (1992). Infectivity and effectiveness of different species of arbuscular mycorrhizal fungi in micropropagated plants of Mr S 2/5 plum rootstock. *Agronomie* 12, 825-829.
- GONZALEZ, J.A., ESTEVEZ-BRAUN, A., ESTEVEZ-REYES, R. & RAVELO, A.G. (1994). Inhibition of potato cyst nematode hatch by lignans from *Bupleurum salicifolium* (Umbelliferae). *Journal of Chemical Ecology* 20, 513-524.
- HACKENBERG, C. & SIKORA, R.A. (1994). Influence of temperature and soil moisture on the biological control of the potato cyst nematode *Globodera pallida* using the plant-health-promoting rhizobacterium *Agrobacterium radiobacter*. *Journal of Phytopathology* 142, 338-344.
- HAYDOCK, P.P.J. & EVANS, K. (1998). Management of potato cyst nematodes in the UK: an integrated approach? *Outlook in Agriculture* 27, 253-260.
- HAYMAN, D.S. (1983). The physiology of vesicular-arbuscular endomycorrhizal symbiosis. *Canadian Journal of Botany* 61, 944-963.
- HUSSEY, R.S. & RONCADORI, R.W. (1982). Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. *Plant Disease* 16, 9-14.
- IBRAHIM, S.K., MINNIS, S.T., BARKER, A.D.P., RUSSELL, M.D., HAYDOCK, P.P.J., EVANS, K., GROVE, I.G., WOODS, S.R. & WILCOX, A. (2001). Evaluation of PCR, IEF and ELISA techniques for the detection and identification of potato cyst nematodes from field soil samples in England and Wales. *Pest Management Science* 57, 1068-1074.
- INGHAM, R.E. (1988). Interactions between nematodes and vesicular-arbuscular mycorrhizae. *Agriculture, Ecosystems and Environment* 24, 169-182.
- JANOS, D.P. (1980). Mycorrhizae influence tropical succession. *Biotropica* 12, 56-64.

- JONES, P.W., TYLKA, G.L. & PERRY, R.N. (1998). Hatching. In: *The Physiology and Biochemistry of Free-Living and Plant-Parasitic Nematodes* (eds R.N. Perry and D.J. Wright). CAB International, Wallingford, UK, pp. 181-212.
- KJØLLER, R. & ROSENDAHL, S. (2000). Effects of fungicides on arbuscular mycorrhizal fungi: differential responses in alkaline phosphatase activity of external and internal hyphae. *Biology and Fertility of Soils* 31, 361-365.
- LAMONDIA, J.A. & BRODIE, B.B. (1986). The effects of potato trap crops and fallow on decline of *Globodera rostochiensis*. *Annals of Applied Biology* 108, 347-352.
- LARSEN, J., KJØLLER, R. & ROSENDAHL, S. (2001). Comparison of inter- and intraspecific colonization characteristics of *Glomus mosseae* and *Glomus geosporum* within the roots of *Cucumis sativus*. In: *Knowledge on Population Biology of AMF as a tool for Mycorrhizal Technology* (eds J. Rydlova et al.). Book of Abstracts, Cost 838 Action, Pruhonice-Prague, Czech Republic, 26-29 September 2001.
- LARSEN, J. & RAVNSKOV, S. (2001). Interactions between *Glomus intraradices* and other soil biota. In: *Knowledge on Population Biology of AMF as a tool for Mycorrhizal Technology* (eds J. Rydlova et al.). Book of Abstracts, Cost 838 Action, Pruhonice-Prague, Czech Republic, 26-29 September 2001.
- LINDERMAN, R.G. (1988). Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology* 78, 366-371.
- LOUCHE-TESSANDIER, D., SAMSON, G., HERNANDEZ-SEBASTIA, C., CHAGVARDIEFF, P. & DESJARDINS, Y. (1999). Importance of light and CO<sub>2</sub> on the effects of endomycorrhizal colonization on growth and photosynthesis of potato plantlets (*Solanum tuberosum*) in an *in vitro* tripartite system. *New Phytologist* 142, 539-550.
- MCARTHUR, D.A.J. & KNOWLES, N.R. (1993). Influence of species of vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition on growth, development, and mineral nutrition of potato (*Solanum tuberosum* L.). *Plant Physiology* 102, 771-782.

- MINNIS, S.T., HAYDOCK, P.P.J., IBRAHIM, S.K., GROVE, I.G., EVANS, K. & RUSSELL, M.A. (2002). Potato cyst nematodes in England and Wales - Occurrence and distribution. *Annals of Applied Biology* 140, 187-195.
- NORDMEYER, D. (1990). The search for novel nematicidal compounds. In: *Nematology from Molecule to Ecosystem* (eds F.J. Gommers and P.W.T. Maas). Proceedings of the Second International Nematology Congress. European Society of Nematologists Inc., 11-17 August 1990, Veldhoven, The Netherlands, pp. 281-293.
- OSNUBI, O. (1994). Comparative effects of vesicular-arbuscular mycorrhizal inoculation and phosphorus fertilisation on growth and phosphorus uptake of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) plants under drought-stressed conditions. *Biology and Fertility of Soils* 18, 55-59.
- PERRY, R.N. (1989). Dormancy and hatching of nematode eggs. *Parasitology Today* 5, 337-383.
- PERRY, R.N. (1997). Plant signals in nematode hatching and attraction. In: *Cellular and Molecular Aspects of Plant-Nematode Interactions* (eds C. Fenoll, F.M.W. Grundler and S.A. Ohl). Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 38-50.
- PERRY, R.N., TWOMEY, U., ROLFE, R.N. & WARRIOR, P. (2000). Effects of DiTera<sup>®</sup> on the life cycle of *Globodera rostochiensis*. *Aspects of Applied Biology* 59, 53-58.
- PLENCHETTE, C., FURLAN, V. & FORTIN, J.A. (1983). Responses of endomycorrhizal plants grown in a calcined montmorillonite clay to different levels of soluble phosphorus. I. Effect on growth and mycorrhizal development. *Canadian Journal of Botany* 61, 1377-1383.
- RAWSTHORNE, D. & BRODIE, B.B. (1986). Relationship between root growth of potato, root diffusate production, and hatching of *Globodera rostochiensis*. *Journal of Nematology* 18, 379-384.

- RYAN, N.A., DUFFY, E.M., CASSELLS, A.C. & JONES, P.W. (2000). The effect of mycorrhizal fungi on the hatch of potato cyst nematodes. *Applied Soil Ecology* 15, 223-240.
- RYAN, N.A. & JONES, P.W. (2003). Effect of tuber-borne micro-organisms on hatching activity of potato root leachate towards potato cyst nematodes. *Nematology* 5, 55-63.
- SMITH, G.S. (1987). Interactions of nematodes with mycorrhizal fungi. In: *Vistas on Nematology: A Commemoration of the Twenty-Fifth Anniversary of the Society of Nematologists* (eds J.A. Veech and D.W. Dickson). Society of Nematologists Inc., Hyattsville, Maryland, USA, pp. 461-468.
- SMITH, S.E. & GIANINAZZI-PEARSON, V. (1988). Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 39, 221-244.
- TURNER, S.J. & STONE, A.R. (1981). Hatching of potato cyst nematode (*Globodera rostochiensis* and *G. pallida*) in root exudates of *Solanum vernei* hybrids. *Nematologica* 27, 315-318.
- TWOMEY, U. (1995). *Hatching chemicals involved in the interaction between potato cyst nematodes, host and non-host plants*. Ph.D. Thesis, The National University of Ireland, Cork, Ireland, 128 pp.
- VAN DER HEIJDEN, M.G.A., BOLLER, T., WIEMKEN, A. & SANDERS, I.R. (1998). Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* 79, 2082-2091.
- VESTBERG, M. & ESTAÚN, V. (1994). Micropropagated plants, an opportunity to positively manage mycorrhizal activities. In: *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems* (eds S. Gianinazzi and H. Schüepp). Birkhäuser Verlag, Basel, Switzerland, pp. 217-226.
- WHITEHEAD, A.G. (1991). The resistance of six potato cultivars to English populations of potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*. *Annals of Applied Biology* 118, 357-369.

WHITEHEAD, A.G. (1992). Emergence of juvenile potato cyst nematodes *Globodera rostochiensis* and *G. pallida* and the control of *G. pallida*. *Annals of Applied Biology* 120, 471-486.